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A Decision-Support Tool for Strategic Decision-Making in Biopharmaceutical Manufacture

A thesis submitted to the University of London
for the degree of
Doctor of Philosophy (PhD)

by

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To Jason and my parents for their love and patience

Abstract

The need for software tools to support decision-making relating to biomanufacture is becoming increasingly critical in order to accelerate the time-to-market and reduce costs. The main objective of this thesis is the design and implementation of a decision-support tool that integrates both the business and process perspectives of biopharmaceutical manufacture to aid the evaluation of manufacturing alternatives. The tool, designated *BioPharmKit*, was built on the platform of the simulation package Extend Industrial Suite (Imagine That Inc., San Jose, USA). As an illustration, the tool was used to evaluate manufacturing alternatives for the production of monoclonal antibodies derived from mammalian-based processes. The functionalities of such a tool to model cost summation, perform mass balance calculations, simulate resource handling, and incorporate uncertainties are demonstrated via two industrial-related case studies.

The first case study was based upon the assessment of pooling strategies in perfusion culture of mammalian cells to deliver a therapeutic protein for commercial use. The analysis in this study addressed the trade-offs between investing in a plant with a smaller downstream process (DSP) capacity and employing more frequent pooling of the broth for purification or opting for a plant with a larger DSP capacity and less frequent pooling of broth. The feasibility of each manufacturing option was evaluated based on the annual throughput, resource utilisation profiles and cost of goods per gram (COG/g). Project appraisal was based on expected output values and the likelihood of achieving or exceeding critical threshold indicators generated using Monte Carlo simulations. Critical drivers that may affect the decision were identified through scenario analyses to improve the robustness of the decision-making process. In the second case study, the decision-support tool developed was employed to evaluate the economic feasibility of fed-batch and perfusion cultures. The trade-offs between the relative simplicity and high titres of fed-batch systems and the high productivity but greater complexity of perfusion processes were analysed. The study aimed to investigate the relative economics of the two operational modes by examining key performance metrics such as the COG/g and the net present value (NPV). Another major objective of this study was to compare the relative usefulness and limitations of the decision tree and Monte Carlo simulations, which are typical tools used for risk analysis to aid decision-making in situations subject to uncertainty. Although the decision tree analysis provided a simple approach for decision-making based on the expected values of performance metrics, it does not explicitly consider the underlying uncertainty in each contributory estimate. The Monte Carlo simulation method was more time-consuming but provided a more complete estimation of process uncertainties subject to fluctuating product titres and process yields.

The examples illustrate the benefits of using the tool to investigate the cost effectiveness of different manufacturing alternatives and may assist the process of decision-making in the context of both business and process drivers. It is envisaged that such a tool might be employed in early process development, hence contributing to transparent planning and project management decisions.

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A. C. Lim

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Abbreviations

ADRs	Adverse drug reactions
ATF	Alternating tangential flow
BPS	BioProcess Simulator
cGMP	Current Good Manufacturing Practices
CHO	Chinese hamster ovary
CIP	Cleaning-in-place
CMO	Contract manufacturing organisation
COG(/g)	Cost of goods (per gram)
DSP	Downstream process
EBA	Expanded bed adsorption
<i>E.coli</i>	Escherichia coli
FDA	Food and Drug Administration
GUI	Graphical User Interface
IND	Investigational new drug
IRR	Internal rate of return
Mab(s)	Monoclonal antibody(ies)
NCF	Net cash flow
NDA	New drug application
NPV	Net present value
PBA	Packed bed adsorption
pGH	Porcine growth hormone
PV	Present value
QA	Quality assurance
QC	Quality control
RAD	Rapid Application Development
ROI	Return on investment
SIP	Sterilising-in-place
SOPs	Standard operating procedures
t-PA	Tissue plasminogen activator
USP	Upstream process
WFI	Water for injection

CHAPTER 1

Scope and Background

1.1. Introduction

The biopharmaceutical industry faces enormous pressures to achieve timely delivery of drugs to the market whilst reducing costs. As production technologies for new drugs become progressively more complex, seamless process development is critical to enhance manufacturing operations in order to streamline timelines and reduce cost of goods (Bryom, 2000). Early planning of process development activities, recognition of lead times and efficient resource utilisation can help to achieve these objectives. As a consequence, the application of computer-aided design tools to facilitate process design and development of biochemical processes is of increasing interest. However, to date, the technologies to deliver such potential improvement are not leading-edge (Norris, 2001). They are utilised and deployed more aggressively in process industries such as chemicals, petrochemicals, polymers etc but remain considerably underdeveloped for the description of biotechnology processes. This thesis explores the possible creation of a software tool to model the manufacture of biopharmaceuticals in order to support the decision-making processes in the biotechnology industry.

The aim of this introductory chapter is to provide an overview of the biopharmaceutical industry with its key manufacturing concerns, highlight the role of process modelling and update its current status in the bioprocessing industry. Section 1.2 gives a brief description of the biopharmaceutical drug development pathway. The prime problems in the biopharmaceutical drug manufacturing process are addressed in Section 1.3. The challenges and benefits of bioprocess simulation as well as some state-of-the-art computer packages for simulating biotech-manufacturing processes are examined in Section 1.4. The methods used for cost analysis and several examples found in published literature are discussed in Section 1.5. Techniques for performing risk assessment to incorporate process and market-related uncertainties are provided in Section 1.6. The objectives of this research are highlighted in Section 1.7. Finally, the organisation of the remaining thesis is covered in Section 1.8.

1.2. Biopharmaceutical Drug Development

The beginning of the latest era of biotechnology can be traced back to the mid-1970s with the development of genetic engineering and hybridoma technology. Over the years, advances in both molecular science and technology have enabled scientists to clone genes, express the corresponding protein in bacterial, insect or mammalian cells and for engineers to purify the resulting product. These biotechnological innovations have helped generate a vast number of therapeutic biological products to intervene in diseases and have lead to the foundation of the biotechnology industry. The biotechnology industry has grown substantially since the first biopharmaceuticals were approved in the 1980s (Gosse & Manocchia, 1996) and there are currently 371 biotechnology medicines in development by 144 companies for nearly 200 diseases (Holmer, 2002). The types of molecules under development are mostly recombinant proteins, including growth factors, monoclonal antibodies (Mabs), enzymes, fusion proteins and peptides (Walsh 2000; Lias & Fogerty, 2002). Other drug candidates, such as antisense therapeutics and viral gene therapy agents and vaccines, are also emerging. The major areas of indication are directed at treating arthritis, respiratory disorders and cancer (McNamara, 2002).

Biopharmaceuticals are among some of the most expensive pharmaceutical substances. For example, the sales value of antibodies such as Enbrel and Herceptin is \$4,500/g (Curling, 2000). Since the 1980s, the worldwide sales value of biopharmaceuticals had climbed to US\$5 billion by 1993 (Walsh, 1998). By 1997, the global market value of biopharmaceutical products exceeded the \$7 billion mark (Walsh & Murphy, 2000). The estimated worldwide market value of some notable biopharmaceutical products in 2000 is presented in Table 1.1.

Table 1.1. Estimated annual global sales value of some biopharmaceuticals in 2000^a.

Product	Indications	Year first approved ^b	Sales (\$ million)
Actimmune	Chronic granulomatous disease	2000	8
Activase	Acute myocardial infarction	1996	206
Avonex	Relapsing multiple sclerosis	1996	761
Enbrel	Rheumatoid arthritis	1998	652
Epogen	Anemia (Chronic renal failure)	1999	1,963
Herceptin	Metastatic breast cancer	1998	276
Intron A	Hairy cell leukemia	1997	1,360
Neumega	Thrombocytopenia	1997	34
Neupogen	Neutropenia	1998	1,220
Ontak	Cutaneous T-cell lymphoma	1999	13
Proleukin	Renal cell carcinoma	1998	113
Pulmozyme	Respiratory infections (cystic fibrosis)	1998	122
Remicade	Rheumatoid arthritis	1998	370
Reopro	Anti-blood clotting agent	1997	418
Rituxan	Relapsed, refractory non-Hodgkin's lymphom	1997	444
Roferon-A	Hairy cell leukemia	1999	159
Synagis	Respiratory syncytial virus infections	1998	427

^a Adapted from Ginsberg *et al.* (2002)

^b Source: http://www.fda.gov/cder/biologics/biologics_table.htm

As larger numbers of new biopharmaceuticals progress through the discovery and development phases, more are getting through to the approval phase. Given the undoubted scientific and commercial prominence of this sector, analysts and the biotechnology industry expect the number of approved biopharmaceuticals to grow rapidly. The worldwide biotechnology market is expected to reach a value of \$100 billion by 2010 (Focus on Catalysts, 2003).

1.2.1. The Stages of Drug Development

The drug development process is a lengthy, complex and highly risky one that involves drug discovery, laboratory testing, animal studies, clinical trials and regulatory reviews. Of 5,000–10,000 chemically synthesised molecules screened, only one becomes an approved drug (Pharmaceutical Industry Profile, 1999). The biopharmaceutical companies take extraordinary measures to ensure that a potential drug is safe and effective by subjecting it to a series of tests. A summary of pre-clinical and clinical phases during the drug development pathway is provided in Table 1.2.

Table 1.2. A summary of the testing stages during drug development.

Stage	Test population	Duration	Intention
Pre-clinical	Laboratory and animal testing	3 years	Initial characterisation
Clinical trials			
Phase I	20–80 healthy volunteers	1 year	Determine safety and dosage
Phase II	100–300 patient volunteers	2 years	Look for efficacy and side effects
Phase III	1,000–5,000 patient volunteers	3 years	Monitor adverse reactions to long-term use

The goal of pre-clinical animal studies is to characterise any relationship between increased doses of the drug and toxic effects in the animals. After completing such pre-clinical testing, the developing company submits an investigational new drug (IND) application, detailing pre-clinical findings, methods of manufacture and proposed protocols for initial clinical trials to the relevant authority, e.g. the Food and Drug Administration (FDA) in the USA, for approval to commence clinical trials. Once the drug has been characterised, and even before early clinical trials are under way, the drug is patented by the developing company in order to receive maximum commercial benefit from the discovery (Walsh 1998). Upon completion of clinical trials, the company submits a new drug application (NDA) to the regulatory authority to allow the drug to be placed in the market. After marketing approval is granted, post marketing surveillance is necessary to monitor and assess the safety of the drug.

Once on the market, a drug is taken by many more patients as compared to during the clinical trials. Adverse drug reactions (ADRs) that occur in fewer than 1 in 3,000-5,000 patients are unlikely to be detected in Phase I – III investigational clinical trials and may be unknown at the time a drug is approved (Pharmaceutical Industry Profile, 1999). Biopharmaceutical companies must inform regulatory agencies of serious and unexpected ADRs within 15 days. Companies must also take voluntary corrective steps as appropriate such as withdrawing a drug from the market. While the drug development process may seem lengthy and cumbersome, the biopharmaceutical companies and regulatory authorities have taken stringent steps to ensure safety of all approved medicines.

1.2.2. Time-to-Market

Confronted with competitive pressures of limited finances and tight timelines, biotech companies worldwide are racing to bring new biological products to market. Once the recombinant DNA expression system for a new drug is defined and a patent application submitted, it is essential to complete process development and obtain regulatory approval as fast as possible. Such timely approval and marketing of drugs is crucial in order to gain substantial market share and secure a good profit margin. If the product is not delivered on time to the market, the company may lose share to

competitive products, which can result in a considerable loss of sales revenue. A day's delay in gaining regulatory approval and product availability could be worth approximately \$1 million in lost sales (Clemento, 1999). In addition, the product lifetimes of biopharmaceuticals are shrinking due to intense competition, which leads to reduced earnings in an era with increased product testing, licensing, and operating costs (Gerson *et al.*, 1998). It is imperative therefore that the biopharmaceutical companies accelerate the time-to-market while reducing the costs of development in order to maintain attractive economic returns.

Current estimates for the time-to-market of a biopharmaceutical drug fall in the range of 5 to 12 years, with an average of 7 to 8 years (Foo *et al.*, 2001). Most of this time is spent in the clinical phases (Marks & Power, 2002). Table 1.3 shows the mean phase lengths for biopharmaceuticals over three time periods. The license application review period has shortened over the years but longer clinical phases due to the need for complex and sophisticated technologies used to characterise and manufacture biopharmaceutical products are now extending development times. The growing complexity and length of the clinical trials has resulted in increased development costs. In turn the estimated average cost to develop a new drug to the point of marketing approval was US\$802 million in 2000 with the clinical cost representing 50% of this total cost (DiMasi *et al.*, 2003). The biopharmaceutical industry is working together with the regulatory authority to shorten time for clinical trials so as to speed up public access to effective biopharmaceutical medicines (Reichert, 2003).

Table 1.3. Mean total phase lengths for biopharmaceuticals*.

Time period	Total number of approvals	Clinical phase (months)	Approval phase (months)	Total phase (months)
1982–1989	14	32.7	24.0	56.7
1990–1994	15	46.5	21.3	67.8
1995–1999	26	58.6	14.7	73.3

* Adapted from Reichert (2000)

1.3. Biopharmaceutical Drug Manufacturing

The biopharmaceutical manufacturing industry is highly regulated, having to comply with stringent rules and procedures. All aspects of a biopharmaceutical manufacture must follow the most rigorous standards and principles, outlined in publications that document Good Manufacturing Practice (GMP). After the drug has been approved, most regulatory agencies require that the drug product be tested for its identity, potency, quality, purity and stability before it can be released for use. For this purpose, process controls are crucial to ensure that a specific process will consistently produce a product, which meets its predetermined specifications, and quality attributes at various critical stages of a manufacturing process. Quality control (QC), quality assurance (QA) and batch documentation form an integral part of GMP. Adequate in-process testing, thorough process validation and analytical methodologies are required at various stages during product manufacture to evaluate the effectiveness of the manufacturing processes and to demonstrate lot-to-lot consistency (Chow, 1997; Geigert, 1997). Every aspect of biopharmaceutical manufacture is characterised by extensive documentation in order to prevent misrepresentations associated with verbal communication, facilitate the tracking of the history of any batch of product and to ensure reproducibility within all aspects of biomanufacture (Walsh, 1998). These documents involve standard operating procedures (SOPs), specifications for conformation of raw materials, manufacturing formulae, processing and packaging instructions.

The production of new drugs, using progressively more complex production technologies, the wide range of biopharmaceuticals' potency and stability, combined with sociological factors has triggered the development of stringent regulations in the biopharmaceutical industry (Doblhoff-Dier & Bliem, 1999). With an increasing number of international and national regulations, companies bringing new drugs to market face additional burdens of ensuring and improving quality. These are expensive and time-consuming activities. Good regulations are essential to the gaining of public acceptance and to diminish the risks involved, such as through the contamination of products and the subsequent infection of the patient. Thus, GMP quality management should be viewed as a valuable asset during the process of gaining regulatory approval. Such a system also helps to identify problems,

streamline operations by improving communications between organisational structures and reduce process failure and downtime.

1.3.1. Manufacturing Capacity

The growth of the biopharmaceutical industry has increased the need for large-scale and efficient production of new biochemical entities, and has in turned placed mounting pressure on the biomanufacturing industry to cope with this demand. Several blockbuster bio-drugs, especially Mabs, currently in the market are designed as long-term therapies or require large doses and so they must be manufactured in mass quantities and hence require high volumes of biomanufacturing capacity (Lias & Fogerty, 2002). For example, protein-based therapeutics utilised approximately three-quarters of total industry capacity in the year 2000 (Ginsberg *et al.*, 2002). In the year 2001, the biotech industry anticipated shortages in GMP production capacity (Garber, 2001; Morrow, 2001). However, more recently, there has been a lot of controversy about this issue. Ginsberg *et al.* (2002) and Sinclair (2003) provided an analysis of capacity estimates and reported that there may be sufficient biomanufacturing capacity in the market to meet capacity requirements.

Drug shortages have been in the limelight during recent months (Wechsler, 2002), generating complaints from pharmacists. Limited access has aroused public health authority concerns in examining the factors underlying this troubling situation. The FDA identifies numerous factors that contribute to drug shortages with many arising from difficulties encountered during manufacturing. Delays in the time-to-market are often due to deficiencies in manufacturing divisions rather than to the scientific or clinical sections in the biotechnology industry (Fisher & Pascucci, 1996) and this is exacerbated by the fact that the design of production techniques for new drugs has become more complex. Suppliers of bulk active raw ingredients may run into production or packaging problems, leading to production delays. Natural diseases may also hinder raw material shipments. Industry consolidation has further aggravated drug dearths (Weshsler, 2002).

Manufacturers often complain however that production problems are largely due to regulatory compliance policies that plague the availability of drugs and vaccines.

Overly-strict regulatory compliance practices have forced them to revise their production processes, leading to considerable time losses. The demand for GMP biopharmaceutical production is becoming apparent, with more products being developed and commercialised. Access to GMP production capacity is critical to guard against shortfalls in drug development, ensure product availability and prevent delays in market launch. The biotech companies are often in a dilemma as to whether to develop expertise in-house, build and acquire their own plants or to employ outsourced biomanufacturing facilities (Grimster, 2003). Because the drug development cycle is compressed, the companies must build a GMP-compliant plant in time to meet clinical trials and commercial sales within a relatively narrow time frame. Furthermore, building manufacturing capacity represents a significant commitment of capital and resources (Lawlis, 2001). Estimated costs for a large-scale biologics facility with a plant capacity of 100,000L range from \$200 million to \$4,100 million (Ginsberg *et al.*, 2002). On average, it may take 4–5 years to design, build and validate a new biologics manufacturing facility (Molowa *et al.*, 2001).

The effects of financial pressure, increased costs of capital and the delays that confront the biopharmaceutical companies favour contracting out manufacturing by accessing the appropriate type and scale of contract manufacturing facilities (Sherwood, 2003). A company can thereby defer capital costs and take economic advantage by relying on contract manufacturing organisations (CMOs) to make their products (Nicholson & Latham, 1994; Seaver, 1994). Outsourced manufacturing supplies skill and technical expertise for different biological products and production methods that the client company may not have, which in turn allows them to concentrate on their core competences (Byrom, 2000). Therefore, contract manufacturing offers an attractive and cost-effective alternative to in-house production. However, this may not be true for all cases. Analysis of the financial outcomes of using in-house manufacturing or outsourcing for a portfolio of companies revealed that for companies with large and diversified potential products, the benefit of contracting out was relatively small (Nicholson & Latham, 1994). This might be expected since such a company can depreciate gradually the cost of its own plant over a number of projects.

1.3.2. Scale-up of Production Processes

The success of manufacturing is also underpinned by the necessity to produce sufficient quantities of the prospective drug for critical steps such as drug characterisation, pre-clinical and clinical testing and commercial introduction (Clemento, 1999; Savage, 2000). Figure 1.1 depicts the scale-up procedure during a typical drug development process (Welsh, 1998). During the R&D phase, probably only a few milligrams are required for initial product characterisation. This could increase to several grams for pre-clinical/clinical trials. For full commercialisation of the final product, the demand could increase several fold, requiring a quantity between hundreds of grams or kilograms. The goal of manufacturing is to meet product demand, as well as rendering the product safe. The proposed manufacturing processes must therefore conform to the highest safety and quality standards. The rise in product demand commands a synchronised change in process capacity (Foo *et al.*, 2001). Although the increase may be accommodated by incremental changes in manufacturing, significant alterations of processes are not usually viable. Any subsequent scale-up or changes to the production protocol could invalidate antecedent efforts and entail additional proof to establish process and product equivalence. As a result, prior planning for the production during the development track must occur even though with significant uncertainty in terms of likely dose and operating capacity.

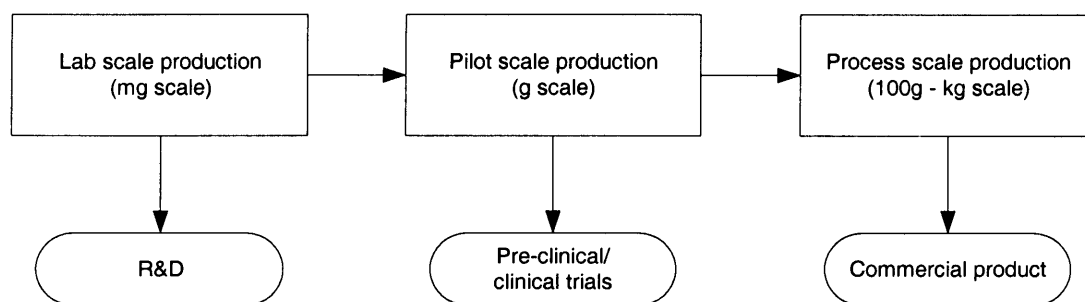


Figure 1.1. Scale-up of biopharmaceutical production process to generate product for initial R&D, clinical trials and commercialisation.

1.4. Process Modelling

Process simulation is one of the most widely used techniques to imitate and analyse the operations of various kinds of real-world processes or systems. The complexities of real-world systems do not allow realistic models to be evaluated analytically and thus, these systems can be studied by means of simulation (Law & Kelton, 1991). Simulation is used to describe the behaviour of a system, ask “what-if” questions about the system and aid in the design of real systems. Simulation models are largely classified along three different dimensions as follows (Banks, 1998).

- *Static vs Dynamic simulation models*

A static model represents a system at a particular time and ignores time-based variances. Dynamic modelling is a software representation of the time-based behaviour of a system.

- *Deterministic vs Stochastic simulation models*

Deterministic models do not contain any probabilistic or random variables. Since all the inputs are constant, the results of simulating a deterministic model are “determined” or set based on the input values. However, many systems typically contain some elements of randomness. Incorporating randomness by adding distributions into a deterministic model changes it to a stochastic model. Stochastic simulations models take into account the effect of variability and provide more reliable estimates of system performance.

- *Continuous vs Discrete simulation models*

In continuous models, values change directly based on changes in time and time changes in equal increments. Discrete-event simulation concerns the representation of a system in which items change state as events occur in the simulation. The simulated time advances from one event to the next and the time between events is unlikely to be equal.

Detailed modelling is required to predict the performance of a system accurately. A simulation study is not a simple sequential process. As new insights about the system of interest are obtained during the study, it is often desirable to revise the

simulation process (Law & Kelton, 1991). The steps intrinsic to a simulation study are illustrated in Figure 1.2.

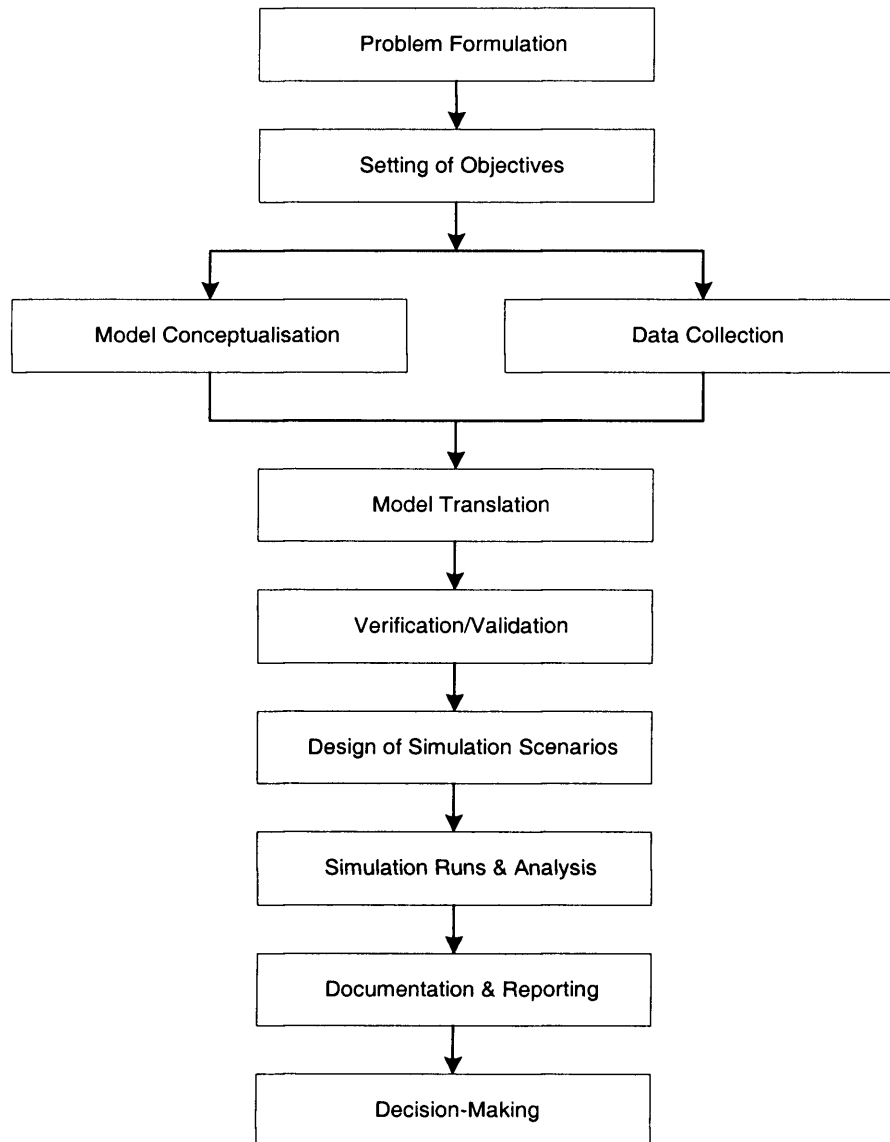


Figure 1.2. Steps in a simulation study. (Adapted from Banks, 1998)

1.4.1. Benefits of Process Modelling

In conjunction with the effort for improved process infrastructure, shortened time-to-market and optimised profits, the use of process design tools to evaluate the feasibility of bioprocess methodologies and procedures is gaining increased awareness (Petrides *et al.*, 1995a). Companies are beginning to recognise the gains of applying such tools to reduce process development time by allowing different processing sequences and operation specifics to be modelled inexpensively. Such tools can be used to screen project ideas and focus on the most promising ones, design process protocols and exploit fully the existing facilities to minimize production cost. Computer-aided process tools also act as a common language of communication among various research and development groups to accomplish the scale-up and commercialisation of biological products.

The key to the successful commercialisation of biopharmaceuticals is to have a well-formulated system involving integration of process design and development at all levels (Petrides *et al.*, 1989). Process modelling enables virtual experimenting or design of possible processes; improve communication between various groups involved in process development; design robust recipe protocols; interpret experimental results and optimise existing plant capacity (Evans & Field, 1989; Petrides, 1994). Simulation can also be used to identify process bottlenecks and determine the cause of delays to improve the time required for process development. Such bioprocess simulations form a framework for process management, resource utilisation, cost analysis, mass balance assessments and prove seemingly useful in the examination and selection of process options, characterization and optimisation of unit operations, which can lead to speeding of biochemical products to market and at optimum profit. The modelling process aids in the design for the manufacturing culture and ultimately delivers a robust and efficient system, which permit better decision-making events in the context of business and process needs.

1.4.2. Challenges in Bioprocess Simulation

Process simulation has been exploited more commonly in the petroleum and chemical industries since it began in the late 1950s. Development of process simulation software for the biotechnology industry has only taken place within the

last 15 years. Due to the complexity of biochemical processes, there is often insufficient data to predict accurately unit operation models based on first principles. Therefore, a bioprocess simulator must rely on empirical and semi-empirical data obtained from experimental and industrial experience. Biopharmaceutical products are manufactured by a series of tasks from inoculum grow-up, fermentation and eventually the concentration and purification of the product. The software tool must be able to model each unit operation with their own specific characteristics to simulate processes of interest. The architecture of the simulator must be flexible to accommodate and accomplish rigorous level of models. The model should handle dynamic allocation of resources, execute cost calculation and perform mass balances. The software platform must provide an interface that enables interaction between the users and the models.

The ability of simulation software to provide accurately a complete model of all phenomena occurring within a biopharmaceutical process is limited (Evans & Field, 1989; Petrides, 1994; Shanklin *et al.*, 2001) due to the lack of fundamental physical property data for biologic systems, as well as the mixing of operational modes employed in a process which can prove difficult to simulate accurately. In addition, ancillary manufacturing operations such as the preparation of intermediate materials, cleaning-in-place (CIP) and sterilising-in-place (SIP) of equipment, scheduling and validation issues must be taken into consideration (Farid *et al.*, 2000; Shanklin *et al.*, 2001). Finally, the administrative management level is often more concerned with the clinical schedule and related business aspects while the production area is faced with tightening deadlines and meeting demands. To achieve a close integration of process and business needs, the software must be able to represent the common information shared between them. These factors were re-emphasised in the recent study of selection of bioprocess simulators for industrial applications (Shanklin *et al.*, 2001). All these problems pose a major barrier in the creation of simulation models that are capable of reflecting real biopharmaceutical processes.

1.4.3. Simulators for the Biomanufacturing Industry

Custom planning methods include general-purpose simulators or spreadsheet-based evaluators, e.g. Excel, equipped with numerical-solving techniques but these methods are often static and limited, leaving a gap between the industrial management and process systems. The use of process simulation software in the biopharmaceutical industry has only evolved during the last 15 years. A review of the literature shows only limited published information on this subject with most of the publications on these topics being from the founders discussing their own software for general bioprocess simulation usage. Two commercial bioprocess simulators namely, BioProcess Simulator (BPSTM) (Aspen Technology, Inc., Cambridge, Massachusetts) and SuperPro Designer® (Intelligen, Inc., Scotch Plains, New Jersey) are used for modelling industrial biotechnology processes. BPSTM, which is an extension of the established chemical process simulator ASPEN PLUS, was the first commercially available simulation tool for the biotech industry. However, BPSTM retained several chemical engineering characteristics that rendered it difficult to evaluate bioprocesses and the simulation tool was withdrawn in 1998. SuperPro Designer® is a subset of BioPro Designer®, which was the second commercial bioprocess tool to emerge.

The use of Aspen BPSTM to model bioprocesses was illustrated by evaluating the production of porcine growth hormone (pGH) from *Escherichia coli* (*E.coli*) (Petrides *et al.*, 1989). Laboratory and pilot plant data were captured in building the flowsheet model to evaluate process economics and improvement. BioPro Designer emerged on the market later (Petrides, 1994). It originated from the Biotechnology Process Engineering Center (BPEC) of Massachusetts Institute of Technology (MIT). The software was further developed and made commercially available by Intelligen, Inc. Until recently, there have been a few publications to provide a critical assessment of these software packages. Researchers at University of Maryland, Baltimore, USA carried out a comparison study between Aspen BPSTM and SuperPro Designer® to evaluate a vaccine manufacturing process under development at Merck & Company (Shanklin *et al.*, 2001). These two simulation packages could successfully perform specific simulation tasks including executing basic material and energy balances; explore equipment change and analyse process

economics. However, such packages do not support dynamic resource allocation to monitor workloads. Furthermore their use is often restricted to built-in models with no option for user-defined models or customisation. An economic evaluation of Aspen BPSTM and SuperPro Designer® for the large-scale production of tissue plasminogen activator (t-PA) from Chinese hamster ovary (CHO) cells was demonstrated (Rouf *et al.*, 2001a). BPSTM is found to be more geared towards chemical processes. As the calculation mode is rigorous, it requires more data to make appropriate use of the package. SuperPro Designer® is more suitable in the absence of detailed data.

Lately, there has been increasing interest in object-oriented based tools for real time and dynamic usage. Research at University College London (UCL) identified Gensym's G2 (Gensym Corporation, Cambridge, Massachusetts), a graphical object-oriented programming environment, to be capable of developing rapid manufacturing prototypes. A tool for modelling strategic decisions in the manufacture of cell-culture-derived products was implemented into ReThink, a G2-based tool (Farid *et al.*, 2000a). A hypothetical case study was evaluated using the tool, involving decision whether to invest in a conventional pilot plant based on stainless steel equipment or to opt for a plant utilising disposable equipment. However, certain aspects of biopharmaceutical manufacturing were not incorporated explicitly into the model such as quality control and documentation activities. Such exclusions might distort the genuine projection of results. Although G2 is a Rapid Application Development (RAD) graphical-interface tool, the average user of the tool must at least possess a certain level of programming knowledge to work with this simulator. This could impair the popularity of the tool in the application of bioprocesses.

1.5. Cost Modelling

The biotech sector tends to focus on getting drugs through clinical trials quickly and as such, minimal efforts are being placed on the development of process technology and manufacturing issues (Pisano & Wheelwright, 1995). However, this is becoming increasingly unacceptable as manufacturing costs escalate. Gura (2002) indicated that the need for cost reductions remain the key issue in the biopharmaceutical

industry. Stringent regulations on product quality and reliability require biotech companies to invest in more sophisticated production equipment and control systems. In addition, the increasing complexity in the design of new compounds commands more advanced and costly production technology. To provide realistic estimates of the value of their technologies, the biotech companies must take into account the cost of producing the product (Stewart *et al.*, 2001). As a result, methods to predict or estimate manufacturing costs are increasingly important in biopharmaceutical companies. Byrom (2000) highlighted that early cost modelling offers an indication of costs at an early stage and identifies process areas likely to give the maximum cost benefits.

Manufacturing costs typically consist of the fixed costs such as the capital charges, insurance and taxes, and variable costs such as raw materials and utilities. The fixed operating costs do not vary with the production rate while the variable costs are dependent on the amount of product produced. Chemical and biochemical engineering textbooks (e.g. Bailey & Ollis, 1986; Peters & Timmerhaus, 1991; Sinnott, 1993) provide the cost estimation breakdown for project evaluation. The production costs are estimated from a flowsheet, which gives the capital cost estimates and raw material requirements. Capital cost estimation is often based on a factorial method. The factorial method is attributed to Lang (1948), where the fixed capital investment is obtained by multiplying the total purchase equipment costs by a factor, usually termed the “Lang factor”. The remaining costs are derived either as a function of utilisation or as percentages of the fixed capital investment or direct operating labour.

Economic appraisals of bioprocess alternatives can be found in the literature. Examples are provided in applications using the commercially available bioprocess tools (e.g. Petrides *et al.*, 1989; Petrides *et al.*, 1996; Rouf *et al.*, 2000). Datar *et al.* (1993) compared the process economics of the production of recombinant t-PA using CHO and *E.coli* fermentations. Costing models were developed to permit the comparative economic analysis to be carried out. The financial parameters used for the comparison purposes include the unit production cost, the gross margin, the cost of sales and the return on investment (ROI). A recent publication involved the use of a financially-based model to evaluate downstream recovery alternatives (Baker &

Wheelwright, 2004). The costing format presented included operating costs as well as the development and implementation costs.

In addition to the production costs, several publications have considered discounted cash-flow analysis to determine the profitability of a project. All cash flows in the future are discounted at a particular rate back to the present to determine the net present value (NPV) of an investment. A positive NPV indicates that the project is worth taking forward. Novais *et al.* (2001) illustrated the use of a NPV analysis to provide an economic comparison of a conventional stainless steel plant and a fully disposable bioprocessing plant for the commercial production of biopharmaceuticals. Other performance indicators that are normally computed together with the NPV are the internal rate of return (IRR) and the payback time. Petrides *et al.* (1995b) provided an example of using such economic indexes to analyse the economic feasibility for the biosynthetic production of human insulin.

The NPV approach has also been widely employed in other engineering situations (e.g. portfolio management) to assess the profitability of investments. Nicholson & Latham (1994) used the NPV analysis to examine the financial impact of in-house and contract manufacturing. Blau *et al.* (2000) described the use of Crystal Ball (Decisioneering, London, England) to generate distributions of NPV for drug portfolio decision-making. Coates (2003) demonstrated the use of the simulation software SLAM II (Pritsker Corporation, Indianapolis, USA) to generate distributions of NPV of a project and compare alternative investment opportunities under uncertainty. The NPV model is a popular method for investment decision. However, the limitation of the NPV model is its subjectivity to discount rates; the slightest estimation error could lead to a huge error in NPV values (Pandey, 2003).

1.6. Risk Management

The complexity of having risks affects the business environment (Nichols, 1994). Biopharmaceutical management is characterised by risks arising from uncertainty in the discovery process, continuing unpredictability in the safety profile of a drug until late phase clinical trials and ultimately during production. For example, common

technical uncertainties in manufacturing systems include process yields, processing times, equipment failures and the possibility of contamination. Other factors include the costs of resources, dosage levels and market demand. Such a high-risk environment often necessitates the need to balance strategic management with risk analysis to improve the quality of the decision-making process.

There are a number of approaches for handling uncertainty. A convenient method is via sensitivity analysis. Sensitivity analysis provides an organised and systematic way to look into the effect of changing parameters on the key output measures. The key input variables in the process were identified and subjected to $\pm x\%$ change (i.e. best-case and worst-case scenarios) while the impact on the output metrics was observed. However, the determination of values that correspond to variations in key factors is based upon the best information at the disposal of the analyst. This inevitably implies the reliance on *ad hoc* methods for determining pessimistic, optimistic and most likely estimates.

For more complicated scenarios, where the probability distributions of uncertain factors are known or may be estimated, Monte Carlo simulation (stochastic modelling) can be used to determine the impact of uncertainties on process outputs. Monte Carlo simulation generates random behaviours for probabilistic factors when applied to a static or dynamic model (Law & Kelton, 1991). Probability description of an input variable is a set of random values that specifies the relative frequency with which an event occurs or is likely to occur. Distributions represent the data observed in real-world situations and help to compensate for information that is often overlooked during data collection. Repeating these simulations many times indicates a range of possible output values and this facilitates the identification of trends or anomalies and helps to determine the expected performance of the system within a certain level of confidence. Farid *et al.* (2001) presented the use of Monte Carlo techniques to assess the impact of risks on the performance of a biopharmaceutical company manufacturing clinical trial materials. Recent simulation studies at Genentech explored the outcomes of adding uncertainties in the planning of manufacturing strategies by using the Monte Carlo technique (Brastow & Rice, 2003). The Monte Carlo model was implemented as a Microsoft Excel add-in. The

model outputs provided probability information about demand distributions and improved insight into the impact of uncertainty in alternative capacity decisions.

The decision tree approach is another useful technique for analysing a decision situation. Decision trees are powerful and simple means to improve project evaluation and planning process. In a decision tree, the expected value of each outcome is computed and a decision is made based on these expected values (Taylor, 1999). The construction of decision trees aids in the understanding of the likely events during the execution of a project and facilitates the calculation of revised probabilities as the project progresses (Raffia, 1968). The graphical illustration of a decision tree proves useful in project selection and management. However, decision trees are not ideal for parallel events that could happen (Doctor *et al.*, 2001). In addition, the construction of a decision tree is a time-consuming process for each project. Sharpe & Keelin (1998) described how SmithKline Beecham constructed decision trees to determine the expected NPV of different portfolios to facilitate resource-allocation decisions. Such expected NPV methodology combines the use of NPV and decision tree analysis by factoring in the probability of distributions of future outcomes and improves the decision-making process.

1.7. Objectives of Research

Global competition to compress timescales and tighten financial budgets is driving the need to improve manufacturing operations in the biotech sector. Hence fast, efficient and effective methods are required to reduce costs whilst simultaneously increasing the speed to market. It is often risky to conduct investigational studies on a full-scale facility. There is a growing demand for tools with which to investigate process strategies in the early development stage of a candidate drug and preferably during initial clinical trial phases (Karri *et al.*, 2001). As noted earlier, computer-aided design processes had long been embraced in the chemical and petroleum industries and are quite advanced in these sectors. However, the use of computer simulation for assisting decision-making in the biopharmaceutical industry is a new area of endeavour. Some large companies have employed a number of software tools, but largely in an isolated and *ad hoc* way. This thesis explores the possibility

of developing a bioprocess simulation support tool in a logical and consistent framework so as to achieve rapid process modelling for the manufacture of biological products.

The modelling framework in this thesis will seek to use the basis set out in previous studies (Farid, 2001) carried out at UCL. It is envisaged that implementation work will need to focus on translating the original framework in ReThink to a Window-based simulation tool and enhancing the tool features so as to facilitate decision-making in biopharmaceutical manufacture. The ultimate objective will be to design a tool for use during the early stage of process development where information is often sparse. The tool can be used to formulate process design methodologies, predict performance of biochemical operations and facilitate decision-support that relates bioprocess decisions to the following strategic issues: costing, resource management and risk. The functionalities of the tool will be demonstrated through its application to industrially-related case studies. In order to achieve such goals, a set of objectives has been identified and discussed below:

- *Integrating business and process perspectives*

In practice, management is often more concerned with the business aspects of cutting costs and can become disconnected from the process issues of tightening deadlines and meeting production demands. In reality these two elements remain quantitatively unconnected and this often results in development that is inefficient. Conventional software tools for bioprocess design have provided limited business-related information for decision-making. These factors have driven the need to capture both the business and process perspectives of biopharmaceutical manufacturing in a bioprocess simulator to facilitate effective decision-making. The conceptual framework seeks to integrate the business and process aspects, including resource management, mass balance analysis and costing, that each relate to strategic bioprocess decision-making.

- *Modelling regulatory-compliance activities*

Manufacturing decisions are often complicated by the need to comply with the ever-increasing demands for regulatory conformation and emphasis on QC/QA. These regulatory compliance activities are critical for controlling the consistency, quality

and safety of biologics. A major challenge faced by biotechnology companies is the need for efficient in-process testing and documentation systems in order to deliver new drugs to the market quickly (Glaser, 2002). An examination of existing process modelling tools for the simulation of biomanufacturing shows that the issue of in-process testing/batch documentation is usually not included in any analysis. Farid (2001) reported the implications of these support activities on the manufacturing environment but no implementation was attempted. This deficiency could vitiate the accuracy of any model to provide an actual reflection of the entire manufacturing process and distort the accuracy of resource management and manufacturing cost estimates incurred. Furthermore including these support activities, which run in parallel with production, is of critical importance in the biopharmaceutical industry in order to identify lead times and bottlenecks in the manufacturing process. As the need for speed to market rises, the application of simulation modelling tools to address these problems is becoming increasingly important. These factors provided the impetus and motivation for this work, which examines the capacity to reflect such regulatory compliance activities in a bioprocess simulator so as to gauge the impact of current Good Manufacturing Practices (cGMP) in biopharmaceutical plants.

- *Incorporating risk analysis*

Commercial process simulators use deterministic models but these do not account for uncertainties and are unlikely to give reliable measures of actual process performance. In reality, production systems typically display some element of randomness. Incorporating randomness into a model can help predict the likelihood of not achieving a certain demand or of the danger of exceeding a financial budget. Not surprisingly there is increasing interest in the ability to model uncertainty in manufacturing processes so as to carry out risk assessments and to estimate the potential risks associated with probabilistic factors during the course of a manufacturing process. The results of such modelling aid understanding of the impact of risks on the process output parameters. The research in this thesis sets out to incorporate such uncertainties in the decision-support tool in order to reflect the inherent variability of process parameters during the operation of a biopharmaceutical plant.

1.8. Organisation of Thesis

This chapter has highlighted the financial and timescale pressures faced during the drug development and manufacturing process, which in part reflect the regulations surrounding the biotechnology industry, and has discussed the challenges of process modelling and available simulators for the biopharmaceutical industry. Cost evaluation techniques for biopharmaceutical manufacture and specific examples found in published literature are discussed. The importance of incorporating risk analysis in processes subject to uncertainty is examined. The objectives of this thesis are identified in the preceding section of this chapter.

Chapter 2 provides a general background to antibodies, their applications and market potential. The various expression and culture methods employed in the industrial manufacture of Mabs are introduced. The analysis given highlights the advantages and disadvantages found in such production techniques and enables typical industrial concerns to be identified, which provide the foundation for subsequent case studies. Generic processes for the commercial production of Mabs expressed in mammalian cells, which are later used as the basis for the unit operations within a biopharmaceutical plant, are defined. Recent trends in the development of commercial antibody production methods are discussed.

In Chapter 3, the suitability of software platforms for the development and configuration of the decision-support tool is outlined. The design methodology and modelling approach for the decision-support tool, designated as *BioPharmKit*, are proposed. The production of Mabs, based on mammalian cell culture, is used for the design, implementation and application of the decision-support tool. The conceptual framework is based on a hierarchical structure in a task-oriented manner. The main components in the domain of the simulation tool are described. Its key characteristics and parameters that relate to both business and process applications are discussed.

An overall discussion of the tool and its utility is provided in Chapter 4. The flow of information during the simulation run is briefly discussed. The key input and output parameters of the tool are identified. A brief discussion of the data collection

methods and the default input values employed to construct and populate the framework is given. The procedures to use the bioprocess tool to assemble and simulate an upstream processing operation are outlined.

Chapter 5 presents an application of the decision-support tool to perform mass balance calculations, simulate resource handling and model cost estimates to aid the evaluation of manufacturing options. A case study is set up to demonstrate the functionalities of such a tool to assess pooling strategies in perfusion culture to deliver a therapeutic Mab for commercial use. A deterministic analysis is initially carried out to determine the base case values. Sensitivity analysis is used to gauge the impact of variables on the key output parameters. The Monte Carlo simulation technique is applied to evaluate potential risks associated with technical and market-related uncertainties. A series of scenarios is considered to investigate their implications on key performance metrics. The statistics derived from the simulation results are then used to determine the ranking of the manufacturing options.

A second case study is presented in Chapter 6 to examine the production economics of fed-batch and perfusion modes of operation. This is achieved by simulating two alternative process flowsheets. The relative economics of the two operational modes are evaluated based on key operational and financial performance metrics. The impact of key influencing factors on the performance measures is investigated via a sensitivity analysis. Both the decision tree and Monte Carlo techniques are employed to incorporate uncertainties such as the risk of contamination and equipment failure. The simulation results from each method are analysed in order to compare each of their performance to compute useful statistics.

Chapter 7 provides a summary of the results achieved in this work and suggests possible extensions for future work. Papers published by the author through the course of this work are attached in Appendix A. Finally, the models used to compute the product mass are given in Appendix B.

CHAPTER 2

Monoclonal Antibodies

2.1. Introduction

Since the development of the hybridoma technology in the mid 1970s, monoclonal antibodies (Mabs) have become an extraordinarily important resource for medical research and are now used extensively as therapeutics, diagnostic reagents and imaging agents. The considerable engineering effort to design and produce antibody-based proteins with high specificity is leading to an increase in both the range and the number of applications in which Mabs can be used successfully. With such developments in the understanding of the nature and function of antibody mechanism, the prospects for further application of engineered antibodies are of great potential. However, a major issue in the development of such antibody-based reagents is the cost of production because Mabs are very expensive to manufacture with the production costs estimated as high as \$1,000 per gram (Gura, 2002). In order for the industry to maintain an acceptable margin on future production, there are significant pressures to reduce manufacturing costs at the commercial scale by 1–2 order of magnitude from \$1,000's per gram to \$10–\$100's per gram (Chadd & Chamow, 2001). This has driven the need to investigate the production economics of Mabs for commercial use. The design and implementation of the decision-support tool (Chapter 3) to facilitate the evaluation of manufacturing options was based on

the production of Mabs. Two industrial-related case studies (Chapters 5 and 6) are presented to illustrate typical issues facing the biomanufacturing industry and the use of the tool in addressing them.

The remainder of this chapter is structured as follows. Section 2.2 provides an introductory background to antibodies and their applications. Their expression and production techniques, as well as typical industrial issues concerning antibody production, are discussed in Section 2.3. The generic sequence for the production of antibodies, which is derived from commercial manufacturing methods for antibody-based products in the market, is presented in Section 2.4. A brief discussion of the recent industrial developments in antibody production is given in Section 2.5. Finally, a summary is provided in Section 2.6.

2.2. Background

The initial Mab therapeutic products launched in the 1980s and early 1990s were a commercial and clinical disappointment. One of the main reasons for failure was the fact that the early Mabs induced an immune response in humans, thereby limiting their therapeutic benefit (Sherman-Gold, 1997; Reichert, 2001). The success of Mabs over the next decade may be attributed to a number of important factors. Now, genetic engineering is used to create humanised Mabs with more human antibody content and less mouse antibody content to overcome the immunogenicity problem (Birch, 1999). Advances in recombinant engineered technologies have also enabled the generation of a wide range of new antibody-based molecules, exhibiting virtually any desired specificity (King, 1998; Walsh, 1998). The great reproducibility of antibodies, coupled with their unique targeting ability, has increased the prospects for further application of engineered antibodies in biotechnology. According to the market analyst Datamonitor, Mabs are the fastest growing therapeutic protein class (Lamble, 2000). Mabs are now used in a variety of clinical circumstances, including diagnostic imaging (e.g. cancer, infectious and cardiovascular diseases) and direct therapeutic purposes (e.g. cancers, inflammatory and infectious diseases, and transplantations). Some typical therapeutic antibodies, their specific indications and approval dates are presented in Table 2.1.

Table 2.1. Typical therapeutic antibodies approved by 2004 and their indications^a.

Trade name/ Generic name	Manufacturer	Indication for use and approval date
Avastin/ <i>Bevacizumab</i>	Genentech	Treatment of patients with metastatic colorectal cancer (February 2004)
Humira/ <i>Adalimumab</i>	Abbot Laboratories	For reducing signs and symptoms and inhibiting the progression of structural damage in adult patients with moderately to severely active rheumatoid arthritis (December 2002)
Xolair/ <i>Omalizumab</i>	Genentech	For adults and adolescents (12 years of age and above) with moderate to severe persistent asthma (June 2003)
Campath/ <i>Alemtuzumab</i>	Millennium and ILEX	Treatment of patients with B-cell chronic lymphocytic leukaemia (May 2001)
Enbrel ^b / <i>Etanercept</i>	Immunex Corporation	Reduction in signs and symptoms of moderately to severely active rheumatoid arthritis (November 1998)
Herceptin/ <i>Trastuzumab</i>	Genentech	Treatment of metastatic breast cancer (September 1998)
Remicade/ <i>Infliximab</i>	Centocor	1. Treatment of Crohn's disease (August 1998) 2. Reduction in signs and symptoms of rheumatoid arthritis (November 1999)
Synagis/ <i>Palivizumab</i>	MedImmune, Inc	Prophylaxis of serious lower respiratory tract disease, caused by respiratory syncytial virus (June 1998)
Simulect/ <i>Basiliximab</i>	Novartis Pharmaceutical Corporation	Prophylaxis of acute organ rejection (May 1998)
Rituxan/ <i>Rituximab</i>	Genentech	Treatment of patients with relapsed or refractory low-grade or follicular, B-cell non-Hodgkin's lymphoma (November 1997)
ReoPro/ <i>Abciximab</i>	Centocor	1. Prevention of blood clots in the setting of high risk percutaneous transluminal coronary angioplasty (December 1994) 2. Prevention of blood clots in refractory unstable angina when percutaneous coronary intervention is planned (November 1997)

^a Source: <http://www.fda.gov/cder>^b Enbrel, a soluble receptor fused to an antibody portion, has manufacturing capacity and dosing requirements similar to that of antibodies.

According to the Tufts Center for the Study of Drug Development, by the year 2001, 10 Mabs had been approved as therapeutics by the US FDA (Reichert, 2001). To date, 17 therapeutic Mabs, comprising of 4 different types (i.e. three murine, five chimeric, eight humanised and one human) have been approved (Reichert & Pavlou, 2004). The global therapeutic Mab market generated approximately \$2.6 billion of sales in 2000, compared to \$1.8 billion in 1999 (Ginsberg *et al.*, 2002). The global Mab market reached \$5.4 billion of sales between 2001 and 2002 (Reichert & Pavlou, 2004). Among all the approved products, infliximab (Remicade) from Johnson & Johnson, indicated for Crohn's disease and rheumatoid arthritis, is the best-selling product with sales of \$1.6 billion, accounting for 30% of the total Mab market sales in 2002 (Reichert & Pavlou, 2004).

The estimated market demand, selling prices and dosage level for some typical Mabs are tabulated in Table 2.2. Mabs for diagnostic purposes may require a scale of tens to hundreds of grams per year. Therapeutic antibodies were initially used in limited markets for acute indications but they are now being developed for chronic indications in large markets (Grimster, 2003). This implies that tens to hundreds of kilograms of active drug substance may be required for a single Mab product per year in the market. Due to the large therapeutic doses required and the chronic nature of the diseases that Mabs treat, they represent a large potential market and demand a significant production capacity. This pressure on capacity is further complicated by the number of Mabs presently under development. With more than 130 such products in the clinical pipeline, as many as 16 Mab products are predicted to reach the market between 2004 and 2008 (Reichert & Pavlou, 2004). The value of the global Mab market is projected to increase to \$16.7 billion in 2008 (Reichert & Pavlou, 2004). Furthermore Mabs only represent approximately 25% of the total recombinant molecules in the biopharmaceutical development pipeline. The remaining 75% in the drug pipeline could put an additional strain on current manufacturing capacity (Lias & Fogerty, 2002).

Table 2.2. Estimated selling price, dosage level and market demand for some commercial Mabs^a.

Product	Selling price (\$/g)	Dose/patient (g)	No. of patients	Demand (kg) ^c
Campath	2,400 ^b	0.75	21,000	16
Enbrel	5,214	2.5	100,000	250
Herceptin	5,811	3.5	26,000	91
Remicade	7,227	1.8	100,000	180
ReoPro	56,430	0.023	300,000	7
Rituxan	5,000	4.3	60,000	255
Synagis	14,301	0.14	138,000	20

Sources:

^a Molowa *et al.* (2002)^b Dynamic chiropractic (2003)^c The estimated market demand was based in 2002 (except for Simulect and Zenapax, where the values indicated the demand for 2001).

2.3. Production of Monoclonal Antibodies

The market for protein-derived products, especially Mabs, has grown significantly in the past decade and continues to accelerate at a rapid rate (Gura, 2002). Mabs have long been considered as prospective drug candidates for diagnosing and treating a wide spectrum of diseases. As more recombinant therapeutic proteins enter development and get through the approval phase, more efficient large-scale production of such proteins is necessary to meet the surging demand (DePalma, 2002). The challenge facing the drug companies is to develop or enhance manufacturing capabilities during the early stage of process development. The requirements of the production system depend on the form of the antibody needed and the intended application. For instance, the production of Mabs for use in humans requires more stringent controls of the production process to achieve highly purified

antibody compared to those used for *in vitro* applications (King, 1998; Matejtschuk *et al.*, 1998). Therefore, it is important to identify the particular manufacturing system at the beginning of a project.

2.3.1. Antibody Expression Technology

There are various expression systems that can be used for the production of recombinant proteins. These include bacterial or mammalian cell cultures and transgenic hosts. The choice of expression depends on the intended use of the antibody and the antibody yield obtained from each system (Chadd & Chamow, 2001). Mammalian cell culture is a mature technology and is the most common choice for the production of complex macromolecules such as antibodies (Hu & Aunins, 1997; Kelly, 2001). The majority of antibody products currently on the market are produced by recombinant technology expressed in mammalian cell culture using either Chinese hamster ovary (CHO) or mouse myeloma (SP2/0 and NS0) cell lines (Chadd & Chamow, 2001). Almost all are secreted in their native form into the cell culture fluid (Werner, 1994). Such mammalian cells enable highly effective amplification and expression of recombinant genes. Expression levels are highly variable from one cell line to another. Typical antibody yields from hybridoma cells are between 50–500mg/L (Brown *et al.*, 1992). NS0 cell lines are capable of producing antibody titres between 1–2g/L (Bebbington *et al.*, 1992; Bibila & Robinson, 1995). CHO cell lines have been reported to accumulate antibody yields greater than 550mg/L (Brown *et al.*, 1992). Factors affecting antibody yields include the culture medium used, stability of the cell line, pH, temperature, and feeding strategy (Macmillan *et al.*, 1987).

Both transgenic animals and plants provide an alternative route for the expression of recombinant proteins (Larrick & Thomas, 2001; Andersen & Krummen, 2002; Houdebine, 2002). High protein expression levels can be achieved in such host systems. Transgenic sheep, goats and mice are being investigated for their ability to produce large volumes of milk containing the expressed protein. Antibody expression levels of 1–25g/L in the milk of goats and mice, being 10-fold higher than those in CHO culture systems, have been achieved (Young *et al.*, 1998a).

Increasing interest in the production of recombinant proteins in the milk of transgenic animals, the eggs of transgenic chickens and in transgenic plants can be attributed to claims of a potentially lower cost of goods compared to mammalian cell cultures (Young *et al.*, 1998b). The cost of raw materials is estimated at \$150/g for mammalian cells and \$1–2/g for transgenic goat milk (Dove, 2002). Typical cost per gram estimates at different production rates for various expression systems are summarised in Table 2.3. The factors affecting the cost of goods of the manufactured protein include the annual production capacity, purification yield and product titre. The trends in manufacturing indicate that the larger the production scale, the lower the cost per gram for a given titre (Berthold, 2002). The cost estimates in transgenic hosts reported in the literature range from \$15–300/g (Walter, 2001) as compared to \$300–3,000/g for Chinese Hamster Ovary (CHO) cell lines (Young *et al.*, 1997; Walter, 2001; Gura, 2002). These results suggest that production in transgenic plants is the cheapest, followed by transgenic goat and then mammalian cell culture.

Table 2.3. Estimated cost of goods per gram for various expression systems at given production rates^a.

Expression system	Cost of goods per gram (\$/g)		
	10 kg/yr	100 kg/yr	1,000 kg/yr
CHO cells	700	300–3,000 ^b	-
Transgenic goats	300	100	40
Transgenic plants	-	50	15

^a Adapted from Walter (2001)

^b Source: Young *et al.* (1997)

Onigman (2000) reported that the investment costs for biopharmaceutical facilities could range from \$70 million to over \$100 million. A summary of the capital investment estimates for various expression systems at different production rates is provided in Table 2.4. These predictions suggest that the capital requirements for mammalian-based systems could be more than double those for transgenic-based processes. The lower capital investment required in transgenic systems could be

attributed to replacing low-cost animals with high-cost traditional fermentation equipment in mammalian cell cultures.

Table 2.4. Projected capital investment estimates for various expression systems at given production rates*

Production system	Capital investment (\$ million)		
	250 kg/yr	500 kg/yr	1,000 kg/yr
Bioreactors	145	205	295
Transgenic goats	75	90	105

* Adapted from Onigman (2000), assuming a titre of 300mg/L for bioreactor systems.

2.3.2. Industrial Choices for Large-Scale Production

The growing demand for antibody-based products has motivated the development of more efficient and reliable mammalian-cell-culture production technologies. Cell culture systems can be carried out in different bioreactors such as packed-cell perfusion bioreactors (e.g. hollow-fibre and ceramic-matrix reactors) and stirred tanks. Recent published literature indicates that the biotech industry has converged on using suspension cultures in stirred-tank reactors as the standard technology of choice for large-scale cell culture production (Chu & Robinson, 2001). Traditional barriers such as the issues of adapting cells to suspension cultures, shear sensitivity and oxygen supply, have been resolved. The relative scalability and uniform growth environment of the stirred tank culture facilitate monitoring and control of operating conditions such as pH, oxygen concentration and temperature. Table 2.5 lists the following data for each approved product from 1996 to 2003: the cell line and the production method. The products are categorised on the basis of their applications (i.e. therapeutic or diagnostic).

Table 2.5. Approved products generated in mammalian cell-culture systems, 1996 to 2003^a.

Trade name	Cell line	Method
<i>Therapeutics</i>		
Xolair	CHO	Suspension culture
Campath	CHO	Suspension culture
Enbrel	CHO	Not revealed
Herceptin	CHO	Continuous perfusion
Remicade	SP2/0	Continuous perfusion - spin-filter ^b
Synagis	NS0	Fed-batch, stirred tank ^c
Simulect	Myeloma	Continuous perfusion
Zenapax	GS-NS0	Not revealed
Rituxan	CHO	Suspension, stirred tank
ReoPro	Myeloma	Perfusion culture
<i>Diagnostic products</i>		
ProstaScint	Hybridoma	Hollow fibre perfusion culture
Verluma	Mammalian cells	Not revealed
Myoscint	Hybridoma	Not revealed

Sources:

^a FDA approval information on <http://www.fda.gov>EMEA authorised products (EPAR) on <http://www.emea.eu.int/htms/human/epar/epar.htm>^b Deo *et al.* (1996)^c Schenerman *et al.* (1999)

Fed-batch and perfusion culture are the two dominant modes of operation for mammalian-cell-culture based processes, especially for the production of recombinant therapeutic proteins and antibodies required in large amounts (Hu & Aunins, 1997). Large-scale cell-culture production processes are typically carried out in stirred fed-batch or perfusion bioreactors (Chu & Robinson, 2001) (Figures 2.1a and b). Therefore, the operation of these technologies is investigated further in

this study. Alternative modes of operation such as batch and repeated-batch processing (Birch & Froud, 1994) will not be considered.

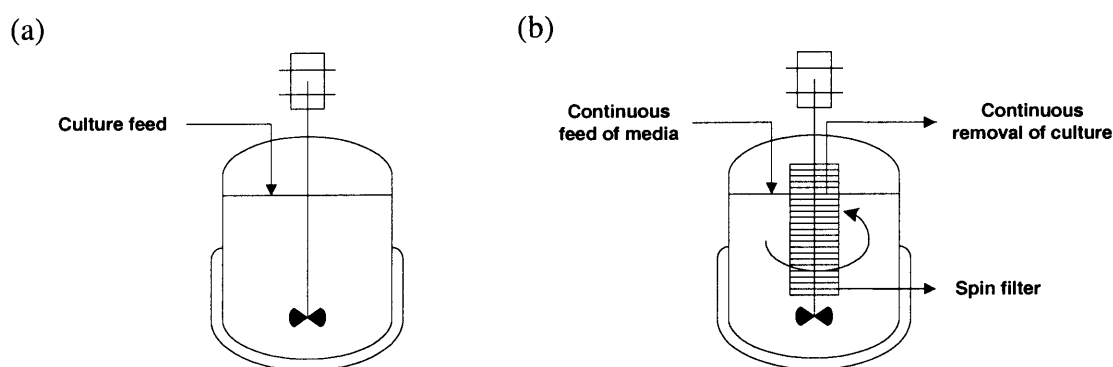


Figure 2.1. Alternative stirred bioreactor processes for (a) fed-batch and (b) perfusion. In stirred tanks, the temperature, pH and dissolved oxygen level are controlled.

In fed-batch culture, there is a gradual addition of fresh volume of selected nutrients during the growth culture cycle to improve productivity and growth (Figure 2.1a). The culture is subsequently harvested and the product recovered. Fed-batch culture has been an attractive choice for large-scale production of Mabs due to its operational simplicity, reliability and flexibility (Bibila & Robinson, 1995) but there has been much interest in perfusion culture in recent years due to the high productivity achieved in such culture systems. In perfusion culture, a continuous supply of fresh media is fed into the bioreactor while growth-inhibitory by-products are constantly removed. The principal aspect of perfusion operation, which is more complex than fed-batch, is the procedure of cell retention. Such a culture mode requires significantly more management, control and maintenance for successful operation. Existing cell retention techniques include cross-flow filters, hollow fibres, spin-filters and vortex-flow filters. Table 2.6 provides a list of perfusion culture data in selected experiments using cell retention by filtration. Woodside (1998) and Voisard *et al.* (2003) compared and evaluated the relative merits and limitations of such retention methods for industrial applications. Voisard *et al.* (2003) reported that

internal spin filters appear to be the best technique in the context of industrial scale manufacturing processes with perfusion rates of 1,000L/day and above. Spin-filter based bioreactors are currently used for continuous industrial production processes (Chu & Robinson, 2001). The operation mode of a perfused culture system in an internal spin-filter based bioreactor is illustrated in Figure 2.1b. The system incorporates an internal rotating filter device in a stirred-tank reactor. The cells are inoculated and cultivated by continuous feed of fresh nutrient medium to the outside of the spin-filter and there is a continuous removal of the product-containing liquor from inside the spin-filter. The rotating spin filter minimises the loss of cells when spent culture is removed/harvested.

Table 2.6. Cell retention by filtration: Summary of perfusion culture data in the literature.

Cell line (system)	Reactor volume, RV (L)	Duration (days)	Maximum perfusion rate (RV/day)	Study
Hybridoma (Spin-filter)	0.55	30	1.36	Reuveny <i>et al.</i> (1986)
Hybridoma (Hollow-fiber)	1.5	32	1.3	Martin <i>et al.</i> (1989)
Myeloma (Spin-filter)	12	14	1	Yabannavar <i>et al.</i> (1992)
Hybridoma (Spin-filter)	175	5	0.5	Yabannavar <i>et al.</i> (1994)
Hybridoma (Hollow-fiber)	0.55	70	2.0	Banik & Heath (1995)
Myeloma (Spin-filter)	500	30	1	Deo <i>et al.</i> (1996)
CHO (Spin-filter)	100	14	0.5	Iding <i>et al.</i> (2000)
Hybridoma (Vortex-flow filter)	1.6	17	4.3	Mercille <i>et al.</i> (2000)

2.3.3. Operational Considerations

The key factors of consideration between the modes of operation include reactor productivity, ease of scale-up and operation, process economics, product stability and up-front investment requirements.

Productivity comparison

Several parameters have been used to evaluate the antibody production performance for different bioreactor processes such as the antibody titre in the broth, the quantity of antibody produced during a defined period and the volumetric Mab production rate. Mahadevan (2003) suggests the volumetric productivity as an optimal metric for cross-bioreactor productivity comparisons. It accounts for the amount of Mab produced, the time required to produce a certain quantity of Mab and the bioreactor volume required to produce the desired quantity of Mab. Table 2.7 summarises the key differences in titre and volumetric productivity between fed-batch and continuous perfusion systems that have been published in the literature.

Table 2.7. Comparative performances of fed-batch and perfusion cultures.

Fed-batch		Perfusion		Reference
Titre (mg/L)	Productivity (mg/L/day)	Titre (mg/L)	Productivity (mg/L/day)	
220	27	400	660	Reuveny <i>et al.</i> (1986)
		40	200 ^a	Bartley <i>et al.</i> (1992)
1,000–2,000	50–140 ^b			Bibila & Robinson (1995)
		400–500	260–330 ^c	Deo <i>et al.</i> (1996)
2,400	105 ^d			Xie & Wang (1996)
2,700	95 ^e			Zhou <i>et al.</i> (1997)

^a The perfusion culture was operated for 35 days and at a perfusion rate of 5 day⁻¹.

^b Mab titres in the order of 1-2g/L are achieved over a period of 2-3 weeks.

^c The productivity was calculated based on a perfusion rate of 1 day⁻¹ and the production phase from day 10 to day 30.

^d The final antibody titre reached 2,400mg/L over a 550h culture.

^e The final Mab concentration reached more than 2.7g/L in 672h.

There have been a number of attempts to increase the viable cell concentration and prolong culture lifetimes in fed-batch processes (Bibila & Robinson, 1995; Xie & Wang, 1996; Zhou *et al.*, 1997). Typical antibody titres in the order of 1–3g/L have been reported using fed-batch cultures of mammalian cells. The cell densities achieved in perfusion culture (10^7 – 10^8 cells/mL) are typically 1 or 2 orders of magnitude higher than for fed-batch modes ($(1-5) \times 10^6$ cells/mL). In some cases it has been demonstrated that continuous perfusion offers an approximately 10-fold improvements in volumetric productivities compared to fed-batch (Bibila & Robinson, 1995; Mahadevan, 2003). Although Mab titres of up to 1–5g/L have been reported (Tyler, 1990) for some perfusion cultures, titres are in most cases lower than those obtained in fed-batch culture due to the continuous dilution of the harvest stream. Published work in the literature indicates antibody titres typically range between 40–500mg/L (Reuveny *et al.*, 1986; Bartley *et al.*, 1992; Deo *et al.*, 1996) for the perfusion culture of mammalian cells. Mahadevan (2003) concluded that perfusion cultures have higher volumetric productivities but lower titre values relative to fed-batch.

Investment considerations

There are numerous fed-batch facilities with reactors in the range of 2,000 to 20,000L. According to the EPAR report (EMEA, 2000) for Herceptin, the cell culture is successively scaled up using a seed train and fermenters from 80L up to 12,000L in a batch/fed-batch operation. Schenerman *et al.* (1999) described the commercial manufacture of Synagis using fed-batch culture in 400, 2,000 and 10,000L stirred tank bioreactors. The fed-batch production of a Mab using NS0 cells was carried out at 8,000L scale (Moran *et al.*, 2000). As a result of the high productivity in perfusion systems, significantly reduced plant capacity is required to produce comparable quantities of antibodies. Deo *et al.* (1996) reported the production of more than 1kg of antibody per week using a 500L continuous perfusion bioreactor and suggested that to produce the same quantity using a fed-batch process would require a 5,000–10,000L bioreactor. The fed-batch system requires a larger reactor volume for a given production rate and hence demands a higher up-front capital investment.

Product stability

The product stability is also a factor influencing the choice of culture mode. An inherently unstable product (e.g. if temporal changes in culture conditions adversely affect product consistency or if product is susceptible to degradation) would require more rapid processing. Perfusion culture may be preferred, because the mean product residence time is in the order of hours instead of days as compared to fed-batch (Kadouri & Spier, 1997; Hoorn, 2000).

Design and scale-up

Fed-batch bioreactor design is a relatively straightforward concept adapted from the batch reactors. As the fed-batch mode is widely adopted for the commercial scale manufacturing of Mabs, typical issues of mixing and parameter controls are well understood. The design of continuous perfusion bioreactors is more complicated due to additional retention devices in the bioreactors (Dalm, 2003). Fed-batch reactors are relatively easy to scale-up while the complexity of perfusion bioreactors poses a greater challenge.

Sterility and technical difficulties

The manufacture of therapeutics based on mammalian cell culture is burdened by the potential risk of introducing foreign agents or inappropriate operating conditions (Hesse & Wagner, 2000). The industry tends to prefer the fed-batch process with its shorter duration and lower risk of contamination. Continuous perfusion processes are subject to an increased contamination risk due to the prolonged operational time. It is difficult to maintain reactor sterility for long periods of time. Possible sources of contamination include improperly sterilised feeds, piping failure and process water systems contaminated with micro-organisms (Jayme & Smith, 2000). The incident of contamination can vary between 10–15% of the batches in continuous fermentations producing kilograms of antibodies (Humphrey, 1996).

Continuous perfusion bioreactors with spin-filters suffer from operational difficulties such as clogging of pores. The problem of filter-membrane fouling has been reported in a number of spin-filter perfusion experiments. For example, filter clogging in perfusion cultures has been reported after 7 days of operation elapsed (Reuveny *et al.*, 1986) and after 14 days (Yabannavar *et al.*, 1992). The fouling of

membrane filters may have a significant impact on the process performance. Filter clogging results in shortened membrane life, increased running costs and premature termination of perfusion cultures.

2.3.4. Typical Industrial Issues

With the growing demand and thriving drugs in the clinical pipeline, manufacturing is an emerging issue in the biotech industry. The key manufacturing challenge is to produce the drug to the correct quality in an efficient and cost-effective manner. This section will highlight typical industrial concerns surrounding the manufacture of antibodies for commercial use.

- *Pooling strategies in perfusion culture*

A major issue in perfusion culture is the need for an efficient pooling strategy in order to utilise fully resources and minimise costs. The options are to invest in a plant with a smaller downstream process (DSP) capacity and employ more frequent pooling of the broth for purification or opt for a plant with a larger DSP capacity with less frequent pooling of broth. The strategy of pooling more frequently provides improved utilisation of downstream equipment due to frequent processing in the downstream equipment and lower investment costs. However, the potential disadvantages include increased equipment-preparation and quality control and assurance costs. Opting for less frequent pooling leads to fewer ancillary equipment-preparation and regulatory-compliance steps but higher fixed overhead costs due to the need for larger DSP capacity and ancillary equipment e.g. buffer vessels. The product stability is also a factor influencing the choice of pooling strategy. A less stable product would require more rapid processing and hence frequent pooling of the broth. Indeed overall little modelling work has been carried out to address such trade-offs in the perfusion mode of operation. The case study analysis in Chapter 5 addresses the trade-offs between these manufacturing options via a computer-aided approach.

- *Fed-batch vs perfusion culture*

Developing large-scale production of mammalian cell products would require a company to choose between the relative simplicity and high titres of fed-batch

systems and the high productivity but greater complexity of perfusion processes. Fed-batch mode of operation typically also involves high start-up costs, owing to the need for a larger bioreactor plant capacity. Increasing interest in the use of perfusion system can be attributed to the high productivity and reduction in reactor capacity (hence easier to operate, clean and sterilise). This results in a significantly smaller plant capacity required to produce comparable quantities of Mabs and allows for a lower initial investment. However, since perfusion processes typically require increased start-up and operational times, equipment failure, including fouling of the device used to retain the growing cells, is more frequently encountered. In addition, with a potentially high number of physical interventions over the extended culture cycle, the possibility of contamination is increased.

Rouf *et al.* (2001b) examined the economic potential of batch and fed-batch operations for the production of tissue plasminogen activator (t-PA) from CHO cells using the simulation package BioProcess Simulator (BPS). It was concluded that although the fed-batch process is more complicated in terms of process operation and control, it offers a significant profit margin over batch culture. Little modelling work however has been done to examine the economics of fed-batch and perfusion systems for antibody production. The case study presented in Chapter 6 seeks to provide an analysis of the running costs and evaluate the trade-offs between these manufacturing options through the use of a computer-aided design tool.

2.4. Generic Antibody Production Processes

The design of a process for the large-scale production of a protein is a crucial step in its successful commercialisation. The process development can pose a significant hurdle with extended lead times. A potential solution is the use of generic purification processes. Figure 2.2 depicts a generic purification process adopted by Amgen. Generic approaches offer the advantages of reducing regulatory issues of processing and shortening development times (Morrow, 2002).

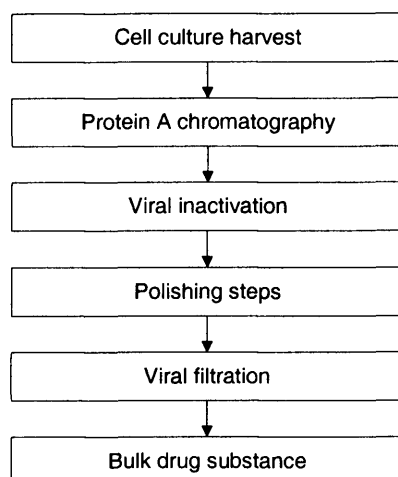


Figure 2.2. Generic purification process for the production of biopharmaceutical proteins, such as antibodies, adopted by Amgen (Adapted from Morrow, 2001).

The proposed flowsheet for the production of a typical Mab by mammalian cell culture, as depicted in Figure 2.3, is a generic sequence derived from an analysis of the production of marketed Mabs (Farid, 2001). The diagram depicts generic manufacturing operations for fed-batch and continuous perfusion cultures. Downstream purification of Mabs produced in mammalian cell lines is simpler than the purification of many other products as the required protein is secreted from the cell in a soluble form. Thus, cellular disruption is not required. The next phase of downstream processing involves removal of the cells and the concentration of the crude product to yield smaller working volumes, which can be subsequently processed with greater speed. At industrial scale, the initial volume can amount to thousands of litres, it is therefore desirable to reduce the process volume at the earliest possible stage for economic and practical reasons. Removal of the cells is achieved by centrifugation or filtration. Filtration is also sometimes used to concentrate the cell-free broth. The clarified broth is then subjected to Protein A affinity chromatography to capture the antibody product and further concentrate it. Viral clearance is followed by further concentration of the broth by ultrafiltration. The concentrated filtrate is then passed through an ion exchange column. Further virus reduction by nanofiltration and concentration of the process stream then takes place prior to gel filtration as a polishing step. A final sterile filtration step is used, yielding an essentially pure antibody preparation.

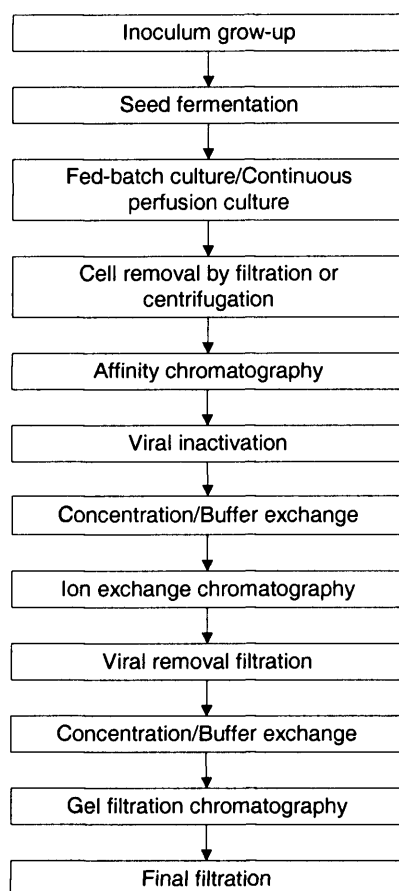


Figure 2.3. Overview of generic antibody production processes based on mammalian cell culture for fed-batch and perfusion cultures. The purification process includes a primary capture step using a Protein A affinity chromatography. Further polishing steps comprise of an ion-exchange-based and gel-filtration-based processes.

2.4.1. Cell Culture

The upstream part is the production of the desired protein in a raw form and includes fermentation/cell culture. Upstream processing commences with the use of a single ampoule to inoculate a few litres of media. After growth, this lab-scale culture is used to inoculate the media in a small bioreactor. This starter culture is then used to inoculate the production-scale bioreactor. The fermentation conditions (e.g. pH and oxygen concentration) are continuously monitored and controlled during the growth process. At the end of the production fermentation process, the crude product is harvested for downstream processing.

2.4.2. Centrifugation

Centrifugation is a unit operation that is commonly used in the downstream processing for the removal of cells and debris from the culture supernatant. Centrifugation is a separation process, which uses the action of centrifugal force to promote accelerated settling of particles in a solid-liquid mixture. It relies on the sedimentation of particles based on the difference in the densities and size of the particle and the medium. Centrifuges come in many designs, with laboratory size handling volumes up to 6L per cycle and industrial scale handling throughputs of up to 150,000L/h (Wheelwright, 1991).

2.4.3. Membrane Filtration

Difference in particle sizes is the driving force in filtration methods to separate the particles from the mixture. The role of the filter is to create a selective barrier through which certain parts of the process stream (the *permeate*) pass through and the remainder of the stream (the *retentate*) is retained. Membrane systems may be operated either in cross-flow mode or dead-end mode. Microfiltration and ultrafiltration operations, which are often used to achieve product recovery and purification, tend to operate in cross-flow mode while sterile-filtration operations are often operated in dead-end mode.

2.4.3.1. Cross-flow filtration

In cross-flow filtration, the flow of liquid is parallel to the membrane with high flowing forces, which enables the suspended solids to be carried away in the cross flow. This reduces the clogging of membrane by sweeping deposits away from the membrane surface. Microfiltration and ultrafiltration are usually operated in the cross-flow mode.

Microfiltration is commonly used to recover cells from the fermentation broth. The larger particles are unable to pass through the pores in the filter and are retained. Microfiltration is generally concerned with particles whose size ranges from about 0.05 μ m to 10 μ m. Typical transmembrane driving forces are in the range of 0.1–2bar while the fluxes of molecules across the membrane are usually more than 50L/m²/h.

With continuous-harvest culture systems, filtration (typically using microfiltration) can be performed simultaneously during supernatant collection.

Ultrafiltration is an effective technique for concentrating or separating molecules of different sizes. Ultrafiltration deals with particles or molecules of approximately 0.001 μm to 0.05 μm . The driving forces in ultrafiltration typically range from 1–5bar. Typical flux values can vary between 10–50L/m²/h. The pores of ultrafilters are small enough to prohibit the flow of proteins through the membrane and allow other components of the process stream to pass through. Ultrafiltration is a popular method of concentration as high product recovery yields and fast-processing times can be achieved.

2.4.3.2. Dead-end filtration

In the dead-end mode, the flow of fluid is perpendicular to the plane of the filter and the particles settle against the filter. Dead-end filtration tends to be employed for sterilising purposes that can occur throughout the process. Its application includes upstream sterility of fermentation broth and as a final filtration step prior to filling.

2.4.4. Chromatography

After concentration, high-resolution chromatographic purification step is usually adopted to purify the protein to a high level. The reduction of process volume can also be achieved by using chromatography purification steps. Chromatography is a separation technique in which the different components in the fluid migrate through the column at different rates. This method consists of interactions between molecules in the sample (fluid phase) and the matrix (solid phase). The matrix is packed in a column. A variety of different chromatography-capture methods are available. There are basically two mechanisms for chromatography: adsorption whereby the molecules are adsorbed onto the chromatographic matrix through a binding process (e.g. affinity and ion exchange chromatography) and non-adsorption process (e.g. gel filtration chromatography). A combination of two to four different chromatographic steps is normally employed during the process.

A typical chromatography step is a batch operation consisting of a load step to feed the inlet stream onto the column, a wash step to remove unbound materials and an elution step to recover separated fractions from the column by the action of an elution buffer. In some cases (e.g. affinity and ion exchange), an additional regeneration step is required to restore the column to a state to which the next inlet stream can be applied. The components in the inlet stream have different distribution characteristics due to their relative affinities for the matrix. As the elution buffer flows through the column, the solutes are resolved and are eluted at different times.

Affinity chromatography is often employed to achieve a high degree of purification. This technique is based on specific interactions between the molecules with a complementary structure bonded to the solid phase that results in a separation from other components in the feed. Ion exchange chromatography is a separation process based on electrostatic interactions between molecules in the sample and the solid matrix. Ion exchange and affinity chromatography steps are both used as concentration methods, which handle large volumes of media and produce a small volume of concentrated product.

Gel filtration chromatography does not rely upon the adsorption of protein to a solid phase and is based on the different rates of diffusion of molecules of different sizes in a porous matrix. Larger molecules traverse the length of a column faster than small molecules. Those molecules, which are small enough to diffuse an appreciable distance into the matrix, slow down in their passage through the column and this results in a separation in time between the components in the inlet stream.

2.4.5. Viral Clearance

Mammalian cell lines may contain viruses that could potentially lead to patient harm. Comprehensive and integrated strategies are necessary to ensure removal of potential virus contaminants in biopharmaceutical production during purification in order to meet the requirements for marketing approval by regulatory agencies (Aranha & Forbes, 2001). Effective viral clearance can be achieved through existing purification steps such as chromatography. In chromatographic operations, depending on the resin and binding capacity, critical contaminants can be eliminated.

Additional steps such as chemical inactivation and virus filtration (e.g. nanofiltration) can be used as effective means of enhancing process viral clearance in manufacturing biological and biopharmaceuticals products beyond those that can be demonstrated by standard protein purification steps.

2.5. Recent Developments in Antibody Production

The previous section has described the current practices for production of commercialised Mabs. However, there are a number of developments emerging, which may in the future be applied to antibody production. Some of these areas are described below. One function of the decision-support tool would be to assess the likely impact of these alternatives.

- *Bioreactor design*

The ATF System[™], introduced by Refine Technology, Co. (East Hanover, NJ) and used in the alternating tangential flow (ATF) mode, is a low shear filtration system that inhibits filter-membrane fouling. Furey (2002) suggested that this external cell-separation system is able to maintain continuous perfusion culture for extended periods of time and offers a rapid change of filters without compromising a culture run. Furthermore, different-sized ATF units are available to scale a perfusion process successfully from the small-development scale to a pilot-scale vessel and a production vessel of 1,000L.

4C Biotech (Seneffe, Belgium) and GSK Biologicals (Rixensart, Belgium) co-developed the sono filtration system for perfused cell culture processes. The device, BioSep, is manufactured and marketed by Applisens (Schiedam, The Netherlands). It relies on an acoustic standing-wave force field to filter the cell suspension. The system does not clog and can be maintained without failure for extended period of time. De Keyser (2002) indicated that quantities in excess of 500g of Mabs have been achieved with productivities and cell densities comparable to that of a tank bioreactor. The process proved to be scalable through the range of units available at 4C Biotech. The BioSep unit has been designed to filter up to 1,000L of cell suspension per day.

- *Disposable/Single-use technology*

As an alternative to the stainless steel reactor, the Wave Bioreactor™, introduced in 1998 by Wave Biotech (Bedminster, NJ), is a presterilised disposable culture system with a scale of up to 500L. This rocking device can be operated in batch or perfusion mode. For the perfusion mode, it is equipped with a floating filter and operated in a wave-induced agitation motion to prevent filter clogging. Singh (1999) has demonstrated its application through the production of monoclonal antibodies using the NS0 cell line from lab-scale of 10L to pilot-scale of 100L. The experiment showed that the bioreactor is well suited for antibody production with comparable performance to stirred-tank bioreactors. Wave Biotech (Singh, 1999) claimed that this disposable reactor is 10-fold cheaper than a comparable bioreactor, due to the elimination of complex instrumentation, and the fact that it does not require cleaning or sterilisation. The Wave Bioreactor has been employed in a perfusion mode for biotechnology applications (Ohashi *et al.*, 2001). In this study, the bioreactor was utilised at a 2L scale to produce a monoclonal antibody based on a hybridoma cell line. The study indicated that the reactor could achieve high cell densities and perform the perfusion operation without clogging for over 25 days. However, the limitation of this system is the accurate controlling of feed media and harvest culture.

The use of bioprocessing plants based on single-use disposable technology appears to be an attractive alternative to conventional stainless steel plants. Fixed stainless steel vessels would be replaced by pre-sterilised disposable plastic containers incurring minimal cleaning costs. Disposable-based engineering could be implemented throughout the production plant, such as the use of disposable filtration membranes, disposable pre-packed chromatography columns and single-use bags for fluid handling. The disposable option has the advantage of switching capital costs to consumables costs instead. The benefits of disposable processing include lower running costs, less downtime and greater process flexibility (Novais *et al.*, 2001; Hodge, 2002; Monge & Hammarberg, 2004). In addition, disposable manufacturing enables facilities to run multiple products in a single plant without the risk of cross-contamination.

- *Transgenic production*

The development of efficient antibody expression techniques is essential for the economic production of highly purified proteins at large scale. Cultured cells have only a limited capacity to produce large amounts of antibodies, even under optimal conditions (Andersen, 2002). Recent advances in the expression of antibodies in transgenic hosts (e.g. goats, chickens and various plant varieties) indicate that these recombinant systems provide alternative platforms to achieve high productivities and low cost protein production. The production of recombinant proteins from the milk of transgenic dairy animals is technically more mature as compared to other transgenic systems (Houdebine, 2002). One of the major advantages of transgenic livestock over cultured cells is their robustness. The levels of milk secretion are very stable and can be maintained constant for weeks or months.

An example of a transgenic livestock application includes antibody production from the milk of goats at Genzyme Transgenics Corporation (GTC) (Young *et al.*, 1998a). Pollock *et al.* (1999) reported that the purification process of a recombinant antibody from transgenic milk at GTC could attain a yield of 65% with a purity of 99.9%. Other advantages of transgenic production include the ability to yield high levels of antibodies (usually in the g/L range), scale-up flexibility since livestock numbers can be increased (or decreased) rapidly and the relative low cost of manufacturing facilities (farms) as compared to more traditional cell-culture plants. This mode of production has the potential to address limitations in capacity and economics, and prove a viable alternative to the large-scale production of proteins. However, existing methods of creating transgenic animals are still relatively inefficient and time-consuming. Technical challenges in the field of biopharmaceutical production in transgenic livestock include improving control over transgenic expression and reducing the effective reproductive time among transgenic animals (Rudolph, 1999). The production of antibodies from transgenic plants may offer similar cost advantages but has other concerns such as environment issues and long timelines to achieve protein availability (Hood *et al.*, 2002; Rosin, 2004).

- *Novel downstream processes*

In the downstream processing of antibody production, the number of unit operations should be minimised to avoid product losses and achieve cost reduction. The

traditional steps involved during the initial purification process are centrifugation, and ultrafiltration, followed by conventional packed bed adsorption (PBA) chromatography (Hjorth, 1997). The development of the expanded bed adsorption (EBA) technology is of particular industrial interest since it allows the purification of the desired protein product directly from the crude feedstock in a single unit operation. Such a technique eliminates the need for initial centrifugation, filtration and purification prior to other high-resolution steps (Figure 2.4). Several attempts have been made to investigate the impact of EBA in antibody production using mammalian cell culture (Fahrner *et al.*, 1999; González *et al.*, 2003). They reported that the yield and purify of the purified antibodies were comparable to those obtained by the standard PBA method. The use of EBA has resulted in a considerably smaller capital investment, reduced running costs and a lower demand for processing time. However, the pitfalls of EBA include more rigorous cleaning protocols and instability due to cell/debris contaminants.

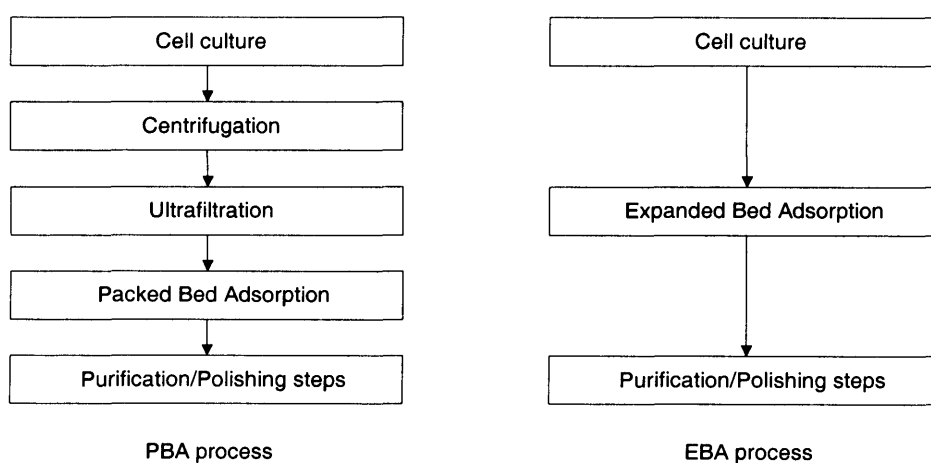


Figure 2.4. Process comparison: Packed bed adsorption (PBA) route versus expanded bed adsorption (EBA) route.

2.6. Conclusions

Mabs play a significant and increasing role in clinical applications as therapeutic, diagnostic and imaging reagents. This chapter has provided an introduction to the production of antibodies, their applications and potential market demand. The

various expression techniques and culture systems for the commercial bulk production of antibodies have been examined. Mammalian cell culture (hybridoma, myeloma or CHO cell lines) is the most common source for the biosynthesis of such recombinant proteins. Reviewing the current processes used in the large-scale cell-culture production of antibodies indicates that the stirred tank bioreactor, with some form of controlled feed (e.g. fed-batch or perfusion), has emerged as the standard technology of choice. The comparative performance and operational considerations of fed-batch and perfusion systems have been presented. Typical industrial issues, which provide the basis for the case studies in this research, are highlighted.

The mammalian-cell-culture production of a typical Mab, based on a generic approach, is analysed. The upstream processing consists of the typical inoculum grow-up operation and seeding fermentation, followed by the production fermentation. The downstream operations comprise primarily of chromatography and filtration steps. Recent trends in the developments of antibody commercial production are discussed.

The proposed conceptual framework, which is presented in the next section of the thesis (Chapter 3), is designed to assist decision-making for biopharmaceutical manufacture and is based on mammalian cell culture. The generic processes for antibody production presented in this chapter provide the foundation for the unit operations included in the tool domain.

CHAPTER 3

Design Methodology and Implementation of BioPharmkit

3.1. Introduction

The manufacture of a drug, in a timely and cost-effective manner, for product testing and product launch can have a dramatic influence on the development timescales and the financial health of a company (Byrom, 2000). Early process planning, improved process yields and efficient resource utilisation are necessary to enhance manufacturing operations. The need for software tools, capable of achieving these objectives, is becoming increasingly critical. The research reported in this chapter sets out to develop a prototype tool, designated as *BioPharmKit*, for application in the production of cell-culture derived products and which addresses key process and business issues in the biomanufacturing sector. The design, implementation and application of the decision-support tool are based upon the production of Mabs expressed in mammalian cells.

This chapter is structured as follows. Section 3.2 describes the manufacturing domain that is addressed by the tool. The scope of the tool framework is indicated in Section 3.3. Section 3.4 provides an assessment of suitable software platforms for the implementation of the decision-support tool. In Section 3.5, the conceptual

modelling framework is proposed. A general overview of the tool structure is described in Section 3.6. The key features and components of the simulation tool are identified in Section 3.7. Finally, a summary is provided in Section 3.8.

3.2. Domain Description

The key issues of the problem domain in biopharmaceutical manufacture are highlighted in this section. Manufacturing issues must be addressed during early process development to ensure sufficient supplies and timely delivery for commercial introduction (Clemento, 1999; Byrom, 2000). At each stage of manufacturing process, validation and process controls are important to assure the drug products meet the standards for safety, quality and stability of biologics (Dobhoff-Dier & Bliem, 1999). However, companies often tend to overlook the importance of staff and resource management within the context of regulatory compliance. Manufacturing development must therefore proceed concurrently with current Good Manufacturing Practices (cGMP) development to prevent pitfalls in procedural control.

The majority of commercial antibody products are manufactured primarily by mammalian cell cultures in stirred-tanks, operated in either fed-batch or continuous perfusion modes. The simulation tool was therefore used to assess the manufacture of biopharmaceuticals based on such processes. The unit operations were derived based on the generic process for mammalian-based production, as discussed in Chapter 2. The resulting protein is usually secreted from the cell. Commercial operations to recover the desired product from the cells include membrane filtration or centrifugation. Downstream purification steps comprise primarily of column chromatography and membrane filtration steps. Viral clearance steps such as chemical inactivation and nanofiltration are added to the process to remove potential virus contaminants derived from mammalian cells.

In addition to the manufacturing unit operations, ancillary activities are involved indirectly with the main production run. These include the preparation of equipment such as cleaning-in-place (CIP) and sterilising-in-place (SIP) operations and

chromatography column preparation steps of equilibration and regeneration. Intermediate materials such as media and buffer solutions need to be prepared prior to the unit operations. Other regulatory support activities include in-process testing steps such as quality control and assurance, and batch documentation.

The process design of a manufacturing operation requires an understanding of the technical process and the operational aspects affecting the business. Table 3.1 illustrates the applications often addressed by both process and business modelling. Process modelling investigates technical performance by developing and specifying the sequence of operating steps and examining the effects on operating variables. Business modelling enhances project management by addressing strategic issues such as project costing and risk analysis. Combining process and business modelling provides a basis to develop the process in a cost-effective manner for commercial production and enhance decision-making in manufacturing.

Table 3.1. Applications of process and business modelling.

Modelling area	Application
Process	Capacity management
	Plant design
	Mass balances
	Allocation of resources
Business	Capital investment decisions
	Cost evaluation
	Cash flow analysis
	Risk assessment

3.3. Scope of Framework

The purpose of the simulation tool was to provide an evaluation of manufacturing options from both the business and process perspectives by capturing process data. The framework should cater for the different unique aspects of the biopharmaceutical-manufacturing domain while maintaining easy simulation usage. The scope of the conceptual framework was defined as follows:

- to prototype biotech manufacturing unit operations and resources,
- to evaluate process economic, time, yield and resource management,
- to perform process data analysis,
- to generate key output data.

The capabilities of the decision-support tool should include the ability to design new processes and evaluate process modifications of existing manufacturing facilities. The simulation model should be able to address the following manufacturing alternatives:

- capacity issues e.g. selecting the most appropriate pooling strategy in perfusion culture given a set of operating constraints,
- process decisions e.g. the use of fed-batch versus continuous perfusion cultures,
- facility design e.g. the use of a stainless steel versus a disposable-based manufacturing plant,
- retrofit decisions e.g. retrofit of expanded bed chromatography technique in a conventional packed bed facility.

The next section of this chapter provides an assessment of the suitability of software platforms for the implementation of the simulation tool. The subsequent sections focus on the description of the approach adopted to model the operational and economic aspects of biopharmaceutical manufacture.

3.4. Assessing Suitability of Software Platforms

The possibility of using a commercially available process simulation package configured specially for the bioprocess industry was investigated in detail by Farid (2001). The study highlighted the pros and cons of using BioPro Designer. The package permits a flowsheet for an entire process to be set up rapidly. It consists of many important default values for input data that can be used when experimental data are not readily available. The key disadvantages of BioPro designer were found

to be the inflexibility to customise and model newer unit operations and the inability to incorporate probability distributions to represent uncertainties in parameter inputs.

Past work at the Advanced Centre for Biochemical Engineering, University College London (UCL), had focused on using ReThink, a graphical application that runs in G2 (Gensym Corporation, Cambridge, Massachusetts), an object-oriented programming environment. The key advantages of ReThink include useful pre-built features that enable extension and customisation for process re-engineering. Such a package facilitates rapid prototyping for process development in a modular and hierarchical fashion by allowing the description of manufacturing activities at various levels of abstraction. However, one of the key disadvantages was found to be that the reporting features of the package were not facilitated. The high cost of a G2 license was also a major consideration factor.

Current research in UCL is looking into the possibility of using a Windows-based software for bioprocess modelling, taking into consideration the benefits of Windows environment features, to replace the capital and programming intensive G2 object-oriented modelling package. This has two distinct advantages. Windows is a very easy domain in which to learn and work and it is the most common environment for engineers in industry.

Extend (Imagine That Inc., San Jose, USA) is a visual, interactive Windows-based simulation package that is tailored for a broad range of industries. Models are constructed graphically by dragging and dropping blocks from library windows onto the model worksheet. Data can be entered directly into block dialogs interactively by using controls or read from files as the simulation runs. The block development environment includes a fully-featured, compiled, ModL language that allows simulation modellers to add customised functionalities. ModL is a relatively easy programming language to learn and program due to its similarity with the C language. This toolkit combines sophisticated statistical analysis with specialised blocks (e.g. processing, batching, transportation) to provide a wide variety of modelling opportunities. It offers unlimited hierarchical decomposition and consists of features to streamline operations, document procedures, identify bottlenecks and plot utilisation profiles. Extend Industry Suite (v5), an extension of the basic Extend,

provides an integrated database system, which is necessary for the storage of process data.

A summary of the assessment of the software packages based on several criteria is given in Table 3.2. On the basis of the above analysis, Extend Industry Suite (v5) was selected as the most appropriate software package and was chosen as the simulation package for the implementation of the decision-support tool. Microsoft Excel was chosen to provide the database interface as it is transparent to most users.

Table 3.2. A summary of assessment of software packages.

Assessment criteria	Software packages		
	BioPro Designer	G2	Extend
Availability of existing unit operations	Yes	No	No
Ease of use of existing process models	Easy	N/A	N/A
Simple v rigorous process models	Only simple	Adaptable to simple and rigorous	Adaptable to simple and rigorous
Ability to create user-defined models	No	Yes	Yes
Availability of numerical-solving techniques	Yes	No (Programming required)	No (Programming required)
Reporting feature	Less convenient (Text file)	Not convenient	Convenient
Cost consideration*	US\$5,500/copy/yr	US\$41,780/copy (Offline version)	US2,995/copy (Extend Industry Suite)
Ability to support stochastic variables	No	Yes	Yes

* Software prices are for industrial licenses as of 2003.

3.5. Modelling Framework

A highly structured bioprocess simulation support tool was proposed in order to achieve rapid process modelling for the manufacture of biological products. The conceptual framework seeks to integrate various aspects, including resource management, mass balance analysis, in-process testing and costing, that each relate to strategic bioprocess decision-making. Figure 3.1 depicts the structured framework of the decision-support tool. The tool proposes the integration of a data bank unit, a code repository unit, a process flowsheet model and libraries of blocks. The diagram illustrates the constant interaction between the manufacturing tasks, resources and the repository of code, allowing mutual transfer of information between them.

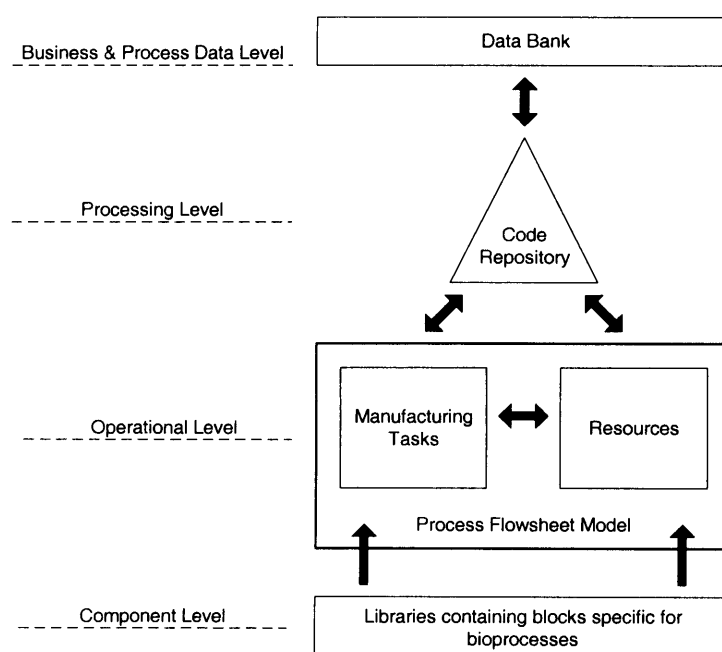


Figure 3.1. A structured framework of the decision-support tool. The framework integrates the business and process data, processing, operational and component levels for rapid bioprocess modelling.

3.5.1. Libraries of Blocks

The decision-support tool contains the toolkit of bioprocess components required for the simulation of the process flowsheet. The components are classified and stored according to their type. The main components of the process flowsheet model are identified as the manufacturing tasks and resources.

3.5.2. Process Flowsheet Model

The biological process steps are modelled in this section of the tool. The user defines the flowsheet for the entire manufacturing process as well as the interaction between the manufacturing tasks and resources.

3.5.3. Code Repository Unit

The code repository unit forms the core of the decision support tool. It enables data retrieval and conveyance of information to the data bank unit, forming an efficient channel of information to the process flowsheet model. This unit also comprises the common functions or procedures initiated by the manufacturing tasks and resources.

3.5.4. Data Bank Unit

In reality, a company would have to analyse a set of process and business input data in order to determine the most effective or workable solution. The data bank gathers and feeds both the technical and management information into the code repository unit. After simulation of the process flowsheet model, output data are sent to the data bank unit via the code repository unit.

3.6. General Tool Structure

3.6.1. Implementation

Having delineated the conceptual modelling framework and selected the software platform, the framework was then translated into a computer-aided tool. The software tool was developed using a task-oriented approach on the platform of the

visual simulation package Extend Industry Suite (v5). Microsoft Excel was selected to provide an interface for viewing results. The tool framework was based on previous work carried out using ReThink at UCL (Farid, 2001). The implementation involved the translation of the original framework to Extend and adding new features to improve the functionalities of the simulation tool. In designing the decision-support toolkit, the main challenge was to build the tool so as to ensure modularity, reusability and extensibility. Specific blocks to describe the bioprocess steps were coded in Extend and linked to represent the whole process within the manufacturing environment. Extend provides a wide range of libraries containing blocks to create models. New blocks were created in the tool to capture features not provided in Extend (Appendix B). The manufacturing processes were configured as time-dependent discrete unit operations. Table 3.3 describes the procedures for translating the framework into the prototype tool.

Table 3.3. Steps carried out to implement the decision-support tool.

Tool element	Procedures
Fundamental structured system	Identify the process/operational levels Create hierarchical underlying structure
Component definition	Identify categories in the manufacturing environment Organise the categories into hierarchies Specify attributes of each category Assign functions to each category Capture same characteristics for subcategories from superior categories
Component inter-relationship	Define interaction between activities and associated resources Determine requirements of activities and resources
Graphical User Interface (GUI)	Create clone items for user inputs Add animation features

The first step was to distinguish and segregate the rudimentary levels of process functions. This was achieved by creating a hierarchical workspace, with each window containing sub-windows. The next step was to identify the different categories involved in bioprocess manufacturing. In this tool, the categories included the unit operations to manufacture the product, supporting activities for the proper operation of the unit operations, and resources required to carry out the manufacturing tasks. Each of these categories was given specific attributes and functions to characterise their features. The interactions between the unit activities and associated resources were then defined. Finally, the capabilities of the tool were enhanced by providing cloned dialog boxes from the coding blocks for users to specify the input values and adding in animation features for visualisation of the simulation process. The tool architecture was continually refined during the design and implementation cycle of this research.

The primary workspace for the decision-support tool is illustrated in Figure 3.2. This workspace contains the *Executive* block for proper tracking of events in the discrete model and a clock for displaying the current simulation time of the model. The workspace includes the sub-workspaces for the code repository, manufacturing template and the libraries for the resource and manufacturing task categories. Each category describes components with the common attributes and characteristics. Each of the workspaces can be decomposed further into lower hierarchical levels revealing more detail. The decomposed engineered platform enabled rapid prototyping of process models in an organised and systematic manner. The code repository contains the functions shared among the manufacturing tasks to manipulate the activities in the model and acts as an interface between the tool and the data bank in Excel. The functions in the code repository included mass balance calculations and cost calculations. The various types of manufacturing tasks and resources were stored in their respective libraries. The declaration of the resource pools and the manufacturing campaign details were placed in the manufacturing template. The product-manufacture, equipment-preparation and regulatory steps were incorporated into the manufacturing recipe windows. The building blocks can be duplicated from the libraries and positioned on the appropriate operational level.

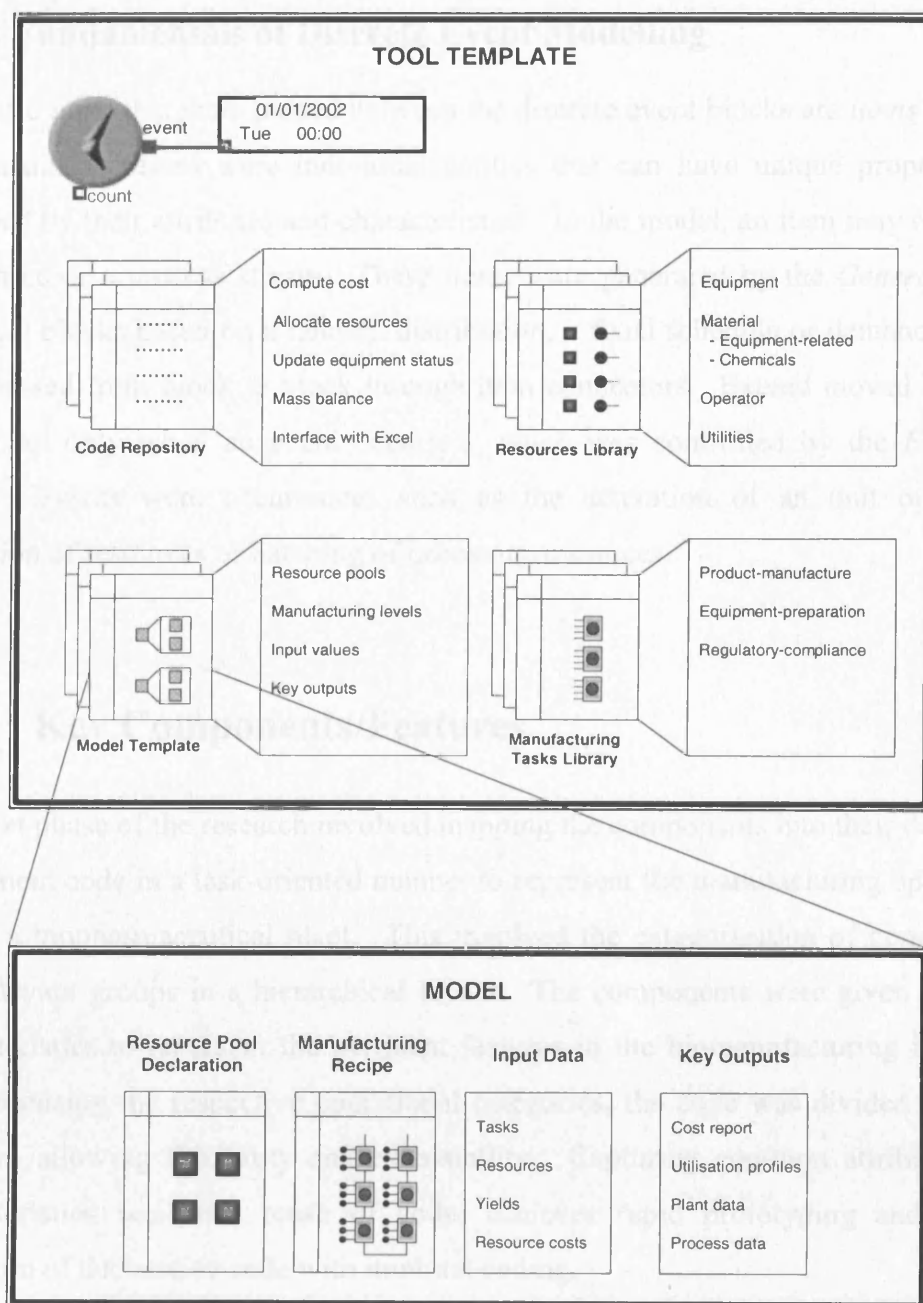


Figure 3.2. A simplified schematic of the prototype template. An executive block keeps track of all events occurring in the discrete model. The tool consists of the code repository unit, the process flowsheet model and the libraries of resources and manufacturing tasks.

3.6.2. Fundamentals of Discrete Event Modelling

The basic units that were passed between the discrete event blocks are *items* (Extend user manual). Items were individual entities that can have unique properties as specified by their attributes and characteristics. In the model, an item may represent a resource or a process stream. These items were generated by the *Generator* and *Program* blocks based on a random distribution, a fixed schedule or demand. Items were passed from block to block through item connectors. Extend moved items in the model only when an event occurred, which was controlled by the *Executive* block. Events were occurrences such as the activation of an unit operation, allocation of resources or batching of necessary resources.

3.7. Key Components/Features

The next phase of the research involved mapping the components into their definitive component code in a task-oriented manner to represent the manufacturing operations within a biopharmaceutical plant. This involved the categorisation of components into relevant groups in a hierarchical layout. The components were given relevant characteristics to represent the pertinent features in the biomanufacturing industry. In customising the respective operational categories, the code was divided out into modules allowing flexibility and extensibility. Capturing common attributes and characteristics facilitates reuse of code, achieves rapid prototyping and allows extension of declarative code with minimal coding.

The key components of the decision-support tool were identified (Table 3.4). The software tool comprised the operational tasks to manufacture a biopharmaceutical (e.g. fermentation, chromatography), additional tasks to prepare the manufacturing equipment (e.g. CIP, SIP), cGMP activities (e.g. quality control (QC)/quality assurance (QA), batch documentation) to test a sample and document a batch, resources required to carry out each task (e.g. equipment, raw materials, utilities, labour), resultant process streams from each task (e.g. media, cells, antibodies-based products, buffer) and cost models. Other key features of the prototype tool consisted

of mass balance calculations, a capacity for process scheduling, dynamic animation, data reporting, and a Graphical User Interface (GUI).

Table 3.4. Key constructs of the prototype tool.

Constructs	Description
Primary manufacturing tasks	Inoculum grow-up
	Seed fermentation
	Production fermentation
	Chromatography
	Viral clearance
	Membrane Filtration
Ancillary manufacturing tasks	CIP
	SIP
	Equilibration
	Regeneration
	Re-equilibration
	QC/QA
	Batch documentation
Manufacturing resources	Equipment
	Operator
	Equipment-related materials
	Chemicals and biochemicals materials
	Utilities
Process stream	Media
	Cells
	IgG
	Buffer
Cost models	Capital investment
	Resource costs
	Cost of goods
	Net present value

3.7.1. Manufacturing Task Category

The manufacturing tasks form one of the key components of the decision-support tool and comprise mainly the *product-manufacture* activities (e.g. *fermentation*, *chromatography*) and ancillary steps of *equipment-preparation* (e.g. *CIP*, *SIP*) and *regulatory-compliance* (e.g. *QC/QA* and *batch documentation*). Each of the unit operations was simulated as an activity requiring resources. The same approach was applied to the modelling of *equipment-preparation* and *regulatory-compliance* operations. The hierarchical levels of the manufacturing activities were realised through the use of workspaces, upon which components can be placed. Each high-level task on a workspace can be broken down into its sub-tasks on their respective sub-workspaces. This is illustrated in Figure 3.3, which shows a *product-manufacture* recipe and an *equipment-preparation* recipe in their respective workspaces. In the process flowsheet workspace, a *Start* block and an *End* block were customised to control the proper simulation of the model. At the start of a simulation, the *Start* block generated an item to characterise the initial process stream. The resultant process stream from the final step of the process exited the *End* block. In this block, the total mass of the antibody-based product generated was updated.

The tool structure was arranged in a hierarchical task-oriented manner to represent the key tasks and resources in a manufacturing operation. Such an approach has recently been employed to model the manufacture of biopharmaceuticals (Farid *et al.*, 2000b). Similar hierarchical decompositions have also been adopted to represent all phases during the process of drug development (Karri *et al.*, 2001; Rajapakse *et al.*, 2004). The framework in this research extended the hierarchy further to incorporate *QC/QA* activities and *batch documentation* (Figure 3.4). As depicted in Figure 3.4, the ancillary steps (e.g. *equipment-preparation*, *QC/QA* and *batch documentation*) were modelled separately from the *product-manufacture* steps. The hierarchical decomposition method proved useful in conferring maximal flexibility since it allows processes to be simulated at various levels of details.

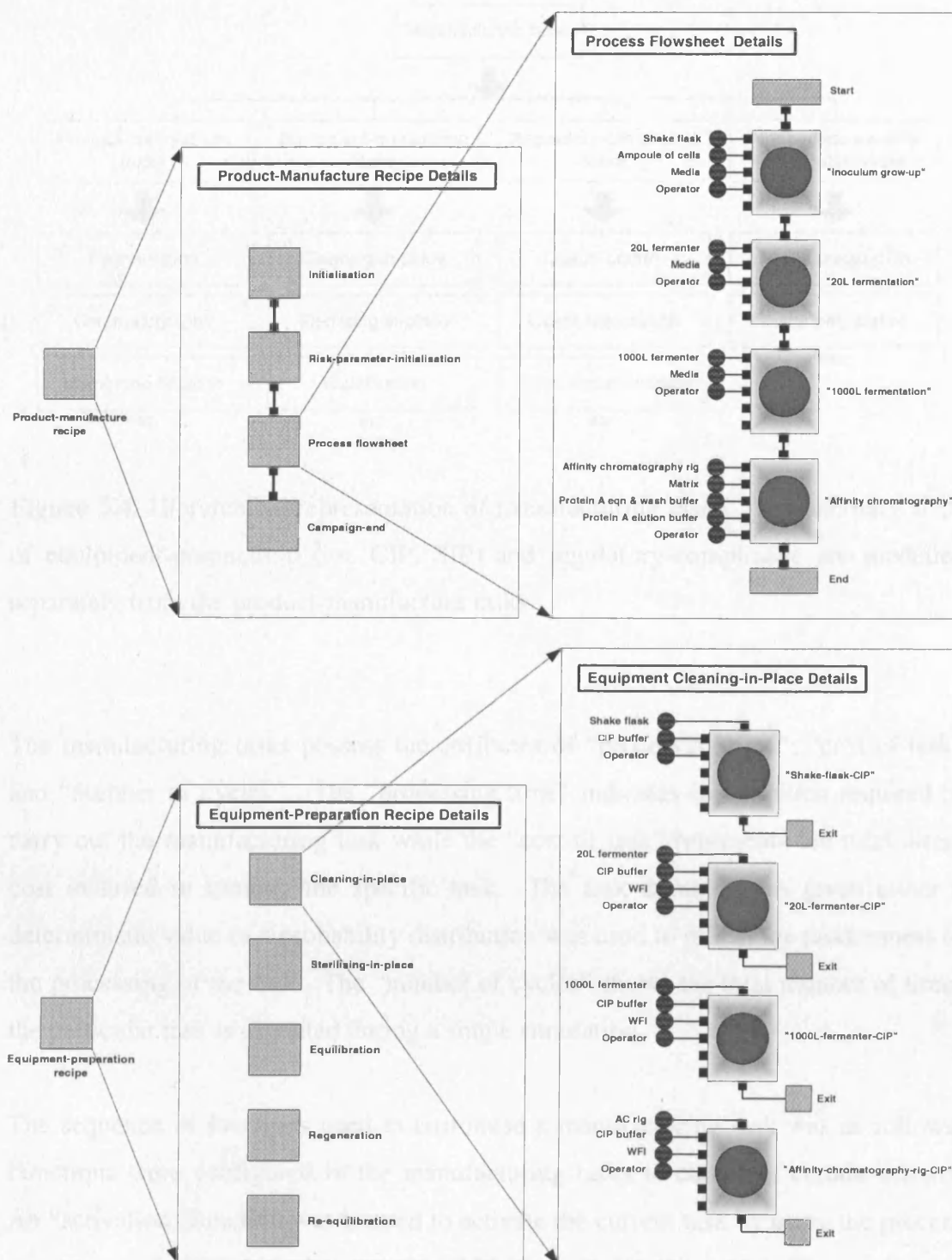


Figure 3.3. Example of a recipe workspace. The diagram depicts a *product-manufacture* recipe and an *equipment-preparation* recipe in their hierarchical workspaces. It consists of a series of product-manufacture tasks as well as related equipment-preparation tasks connected to form part of the biomanufacturing process.

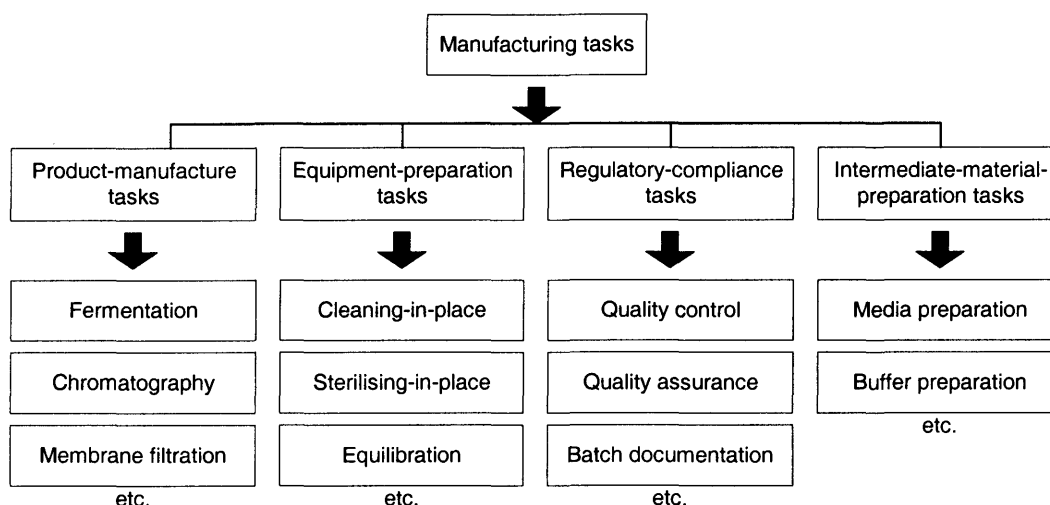


Figure 3.4. Hierarchical representation of manufacturing tasks. The ancillary steps of equipment-preparation (i.e. CIP, SIP) and regulatory-compliance are modelled separately from the product-manufacture tasks.

The manufacturing tasks possess the attributes of “processing time”, “cost of task” and “number of cycles”. The “processing time” indicates the duration required to carry out the manufacturing task while the “cost of task” represents the total direct cost incurred in running the specific task. The task duration was given either a deterministic value or a probability distribution was used to reflect the randomness in the processing of the task. The “number of cycles” shows the total number of times the particular task is executed during a single simulation.

The sequence of functions used to customise a manufacturing task was as follows. Functions were configured in the manufacturing tasks to carry out certain actions. An “activation” function was created to activate the current task by using the process stream item flowing into the customised block from the prior step. This module of code included the *Batch* block to trigger the initialisation of the current block. The function compared the required and current equipment status before commencing the manufacturing process. If the two equipment states do not match, the relevant equipment-preparation tasks were activated. Both the *Throw* and *Catch* blocks provided in Extend were employed. The *Throw* block in a *product-manufacture* task was used to manipulate and activate the *Catch* block in the appropriate ancillary task,

ensuring the correct co-ordination between these two separately modelled manufacturing tasks. In the *equipment-preparation* tasks, the equipment was routed back to the relevant *product-manufacture* task using the *Throw* and *Catch* blocks again. The “activation” function also determined if there were available resources in the resource pool to meet the required demand and to allocate the required resources. The resources were then batched together and delayed by the specified processing time using the *Activity, Delay* block.

After completion of the task, a “cost calculation” function was performed to determine the total direct operating cost. This function used the *DE Equation* block to determine the direct costs of running the manufacturing task. The renewable resources were routed back to their respective resource pools while the non-renewable ones are discarded. The *Throw* and *Catch* blocks were employed to return the resources back to the resource pool and the *Exit* block was used to discard the non-renewable resources. For resources that possess hybrid characteristics, the total number of cycles was coded within the manufacturing task to enable the appropriate computation of cost and when to dispose of the resource. For instance, the cost of chromatography matrices was only added to the operational cost during their first cycle of usage. When their cycle limit was reached, the resource was disposed of. The task block also had a function to automatically update the status of the equipment resource and to invoke the post *equipment-preparation* tasks. For instance, the status of a ‘sterile’ fermenter switches to ‘dirty’ after the fermentation process. The status of a ‘dirty’ fermenter changes to ‘clean’ after the CIP process. Figure 3.5 shows the libraries containing the common tasks in a mammalian cell culture biomanufacturing environment.

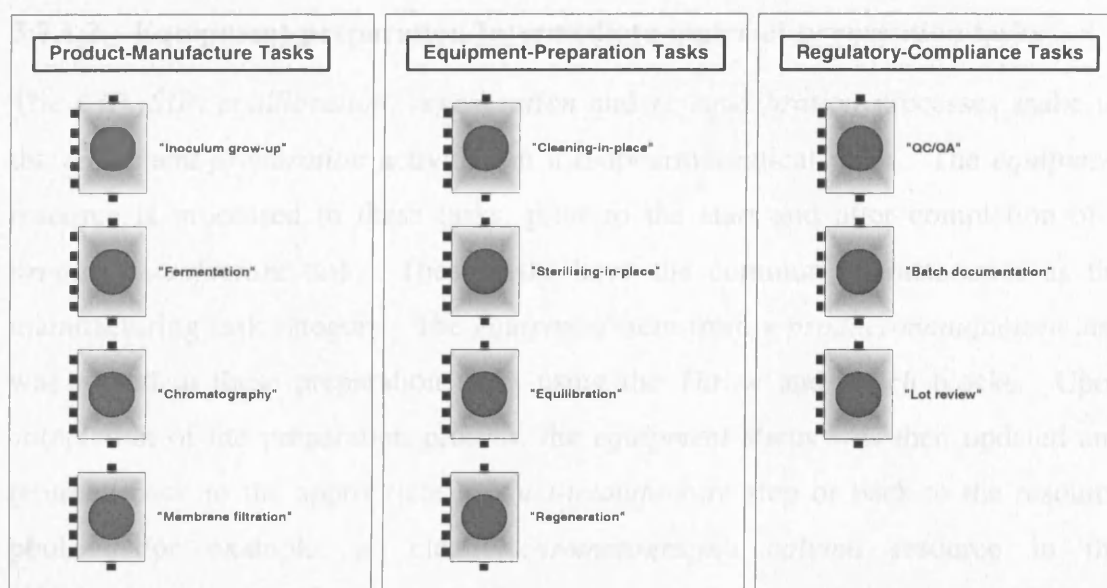


Figure 3.5. Examples of manufacturing tasks in libraries. The task categories include the *product-manufacture*, *equipment-preparation* and *regulatory-compliance* tasks. Any component in a library can be copied and pasted onto the appropriate workspace.

3.7.1.1. Product-manufacture tasks

The *product-manufacture* tasks, such as the *fermentation* and *chromatography* processes, come into direct contact with the antibody-based products. This category of tasks has the same characteristics as the manufacturing task category. In addition to the characteristics of the manufacturing task category, the *product-manufacture* task category possesses a function to perform mass balance calculations during the execution of the task. Appendix B shows the sequence of functions for a *product-manufacture* task and the detail of the "Check Equipment Status" function as an illustration of the coding within each function. Some tasks such as *chromatography* possess a function to determine the duration of the manufacturing steps. A special function is also created to allow the user to specify whether *QC/QA* steps are required for that particular task. In a typical biopharmaceutical plant, quality control and assurance is normally carried out for all major process steps. The default value to carry out *QC/QA* for a *product-manufacture* task is set to true. Since the *batch documentation* steps are carried out for mostly all tasks in the biopharmaceutical plant, they are coded as necessary tasks after finishing the *product-manufacture* tasks.

3.7.1.2. Equipment-preparation/Intermediate-material-preparation tasks

The *CIP*, *SIP*, *equilibration*, *regeneration* and *re-equilibration* processes make up the *equipment-preparation* activities in a biopharmaceutical plant. The *equipment* resource is processed in these tasks, prior to the start and after completion of a *product-manufacture* task. These tasks have the common characteristics as the manufacturing task category. The *equipment* item from a *product-manufacture* task was routed to these preparation tasks using the *Throw* and *Catch* blocks. Upon completion of the preparation process, the *equipment* status was then updated and returned back to the appropriate *product-manufacture* step or back to the resource pool. For example, a ‘clean’ *chromatography column* resource in the *chromatography* process is routed to the chromatography column *equilibration* preparation step. Its status is modified to ‘equilibrated’ and is then allocated back to the *chromatography* step. The status switches to ‘dirty’ at the end of the *chromatography* process step and is routed to the *CIP* task before returning back to the resource pool.

The *intermediate-material-preparation* tasks consist of material preparation activities such as media and buffer preparation. These tasks have the common characteristics as the manufacturing task category. Such tasks are triggered by the *product-manufacture* tasks. Upon activation of the preparation process, the utilisation level of the resource pool for the appropriate resource is adjusted.

3.7.1.3. Regulatory-compliance tasks

Manufacturing decisions are often complicated by the need to comply with the ever-increasing demands for regulatory conformation and the emphasis on QC/QA. These regulatory compliance activities are critical for controlling the consistency, quality and safety of biologics. Existing software packages often omit regulatory-compliance activities in a cGMP-manufacturing plant. Including these support activities is necessary to improve the ability of the model to estimate more accurately operative measures such as costs and resource utilisation. The prototype tool permits the incorporation of such cGMP activities, which are modelled as explicit ancillary tasks in their respective workspace (Figure 3.6).

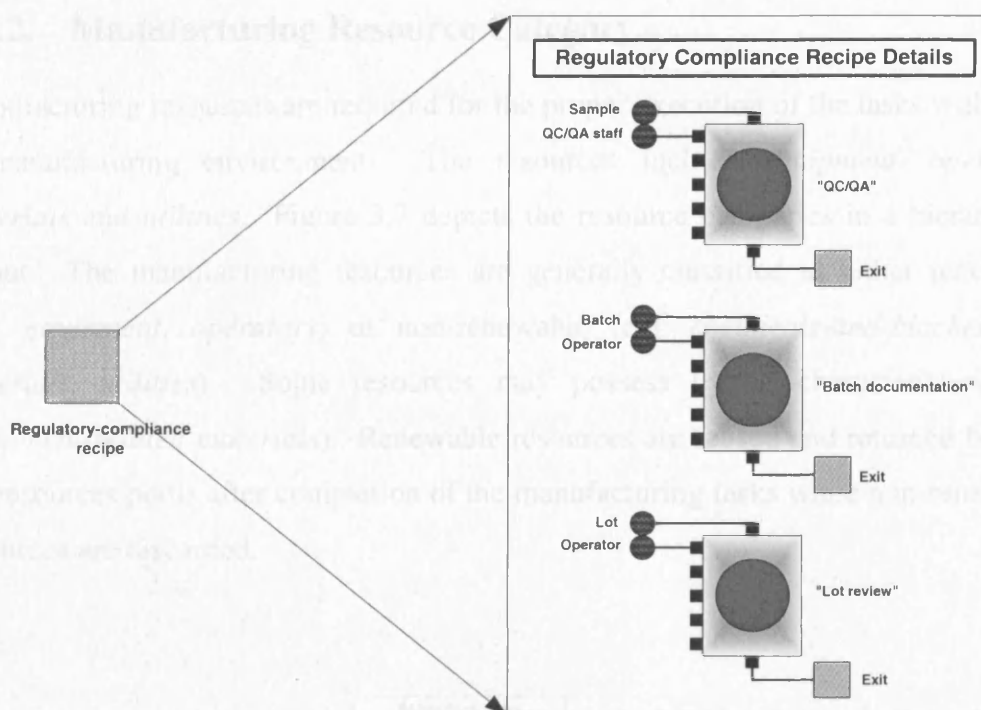


Figure 3.6. A typical regulatory-compliance task recipe. The QC/QA step for all the manufacturing tasks is modelled as one unit operation. The same applies to the modelling of the batch documentation and lot review activity.

The regulatory manufacturing practices within the plant consist of in-process testing, batch documentation and lot review steps. These cGMP activities adopt the same attributes and functions from the manufacturing task category. The *QC/QA* step for all the manufacturing tasks was modelled as one unit operation. The same applied to the modelling of the *batch documentation* and *lot review* activities. The *batch documentation* activities were automatically activated at the end of each *product-manufacture* task while the *QC/QA* steps were triggered based on the user specifications. These *regulatory-compliance* steps were simulated concurrently with the main product-manufacture tasks. In a typical biomanufacturing plant, these regulatory activities are only carried out during specific working hours. A function was configured to customise the scheduling of these regulatory steps for operation only during a particular period. A *Queue, Decisional* block was employed to hold the item during out-of-operating work hours and release the item for processing only within operating hours.

3.7.2. Manufacturing Resource Category

Manufacturing resources are required for the proper execution of the tasks within the biomanufacturing environment. The resources include *equipment*, *operators*, *materials* and *utilities*. Figure 3.7 depicts the resource categories in a hierarchical layout. The manufacturing resources are generally classified as either renewable (e.g. *equipment*, *operators*) or non-renewable (e.g. *chemicals-and-biochemicals materials*, *utilities*). Some resources may possess hybrid characteristics (e.g. *equipment-related materials*). Renewable resources are reused and returned back to the resources pools after completion of the manufacturing tasks while non-renewable resources are discarded.

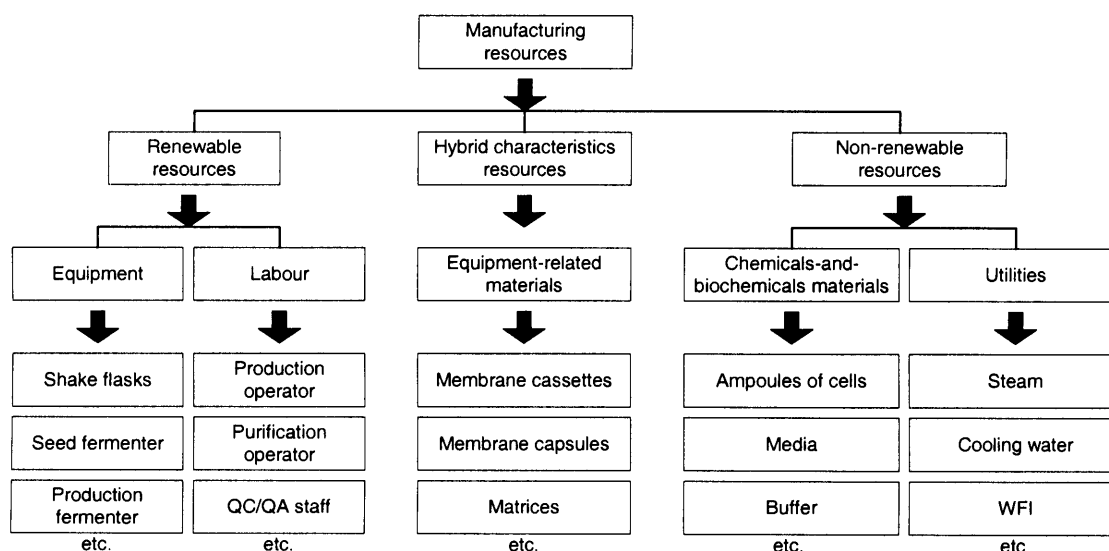


Figure 3.7. Hierarchical representation of manufacturing resources. The resources are categorised into either renewable or non-renewable items. Some resources possess hybrid characteristics.

Modelling of a manufacturing resource was achieved through the combined use of the *resource pool manager*, the *resource* itself and a *queue* block. The *resource pool manager* was given the attributes of “maximum availability” and “cost”. The *resource* possesses the attribute “utilisation”. The “cost” attribute indicates the monetary value of utilising/purchasing the resource. “Maximum availability” refers

to the usage limit of the resource within the plant while “utilisation” indicates the required amount of resources to carry out the manufacturing task properly. These conditions cause constraints to be placed on the simulation flow in the model based on the availability of resources. During a simulation, a resource remains in the queue and is only released when there is a sufficient amount available in the resource pool to meet the requirement.

At the start of a simulation, all the resource pools were initialised to the appropriate usage limit levels. The amount of resources available in the resource pools was managed by the *Global Array Manager* and *Global Array* blocks provided in Extend. All other necessary resources were then checked for availability by utilising these global blocks and batched together to carry out the operation. It was necessary to create a function to adjust the resource pool levels based on the consumption. During the simulation, the amount of resources in the pools was incremented/decremented depending on the type of resources (e.g. renewable or non-renewable). The batched resources were temporarily associated with one another as a grouped item and separated from each other later in the simulation. The resources were then delayed by a time interval equal to the duration of the task. Figure 3.8 illustrates the schematic of the manufacturing resources classified in their respective libraries.

3.7.2.1. Equipment and operators

Besides the characteristics of the renewable resources category, all *equipment* resources possess the attribute “equipment status”. More specific attributes were given to subcategories such as *fermenters* and *chromatography columns* to indicate their sizes. These include “equipment volume”, “equipment height” and “equipment diameter”. A function was created to determine the diameter of equipment based on the values of equipment volume and height. The default value for the initial “equipment status” was automatically set to be ‘clean’. On the other hand, further definitions are not necessary to characterise the *operator* resources. The attribute “cost” was defined as the purchase cost for the *equipment* resource and the wage per hour for the *operator* resource.

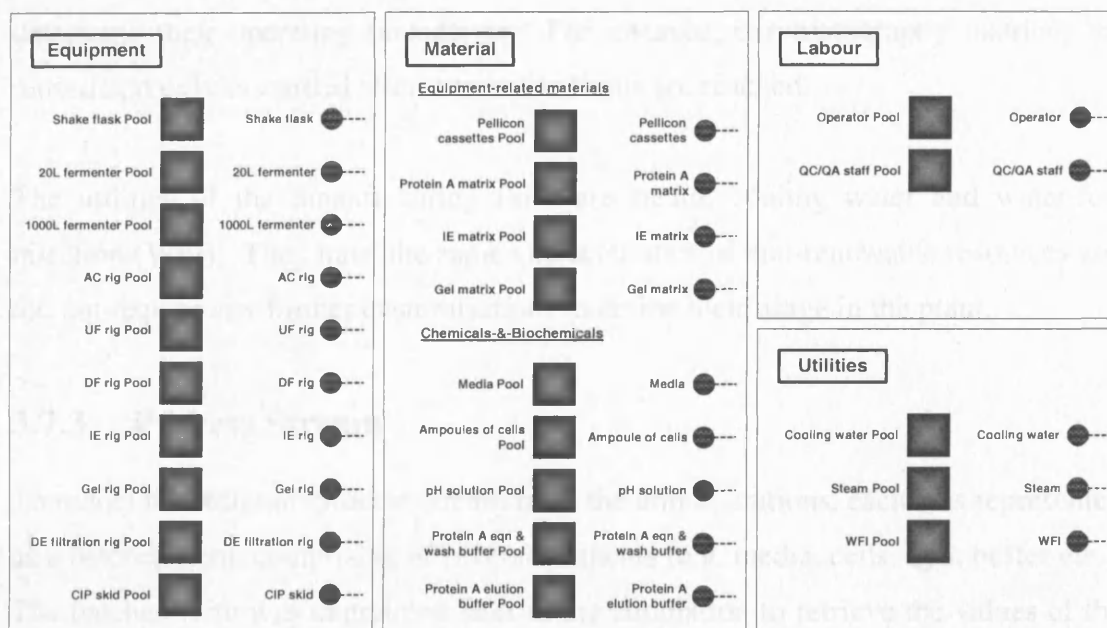


Figure 3.8. Examples of manufacturing resources in libraries. The resource categories include the equipment, material, labour and utilities. The resource pools store the current available number of resources. The appropriate resources are attached to a particular manufacturing task to carry out the operation.

3.7.2.2. Materials and utilities

Material resources were classified into the *chemical-and-biochemicals materials* (e.g. media, cells, buffer) and *equipment-related materials* (e.g. membrane filters, matrices). The former has the same characteristics as the non-renewable resources category. The “cost” is measured as the usage cost per litre. They were given additional attributes such as “mass”, “volume”, “density”, “concentration”, “physical-state” and “virus-titre” to permit the mass balance calculations.

The *equipment-related materials* were categorised as possessing hybrid characteristics. The non-renewable materials (e.g. disposable bags, membrane filters) possess the same attributes and characteristics as the non-renewable resources category. The attribute “cost” is measured as the purchase cost per unit. The attribute “membrane area” is also attached to the filter resources in order to describe their technical performance. The same attributes apply to those *equipment-related materials* having both renewable and non-renewable characteristics (e.g. membrane cassettes, matrices). In addition, they were given the attribute “cycle limit” to

determine their operating boundaries. For instance, chromatography matrices are reused and only discarded after their cycle limits are reached.

The utilities of the manufacturing floor are steam, cooling water and water-for-injection (WFI). They have the same characteristics of non-renewable resources and did not require any further customisations to define their usage in the plant.

3.7.3. Process Stream

To model the resultant process stream from the unit operations, each was represented as a batched item, comprising of several elements (e.g. media, cells, IgG, buffer etc.). The batched item was ungrouped later in the simulation to retrieve the values of the attributes for mass balance. The process stream carried the attributes (e.g. “mass”, “volume”, “density”, “concentration”). Each element in the final product stream was also represented as an individual item possessing its own characteristics (e.g. “mass”, “volume”, “density”, “concentration”, “physical state”, “virus titre”). Such information was then carried over to the subsequent operating task for mass balance calculations.

3.7.4. Mass Balance Calculations

The mass balance calculations were computed using the equations compiled in previous studies (Farid, 2001) to determine the composition of process streams. The process models employed were used to describe manufacturing processes based on mammalian cell culture. Each unit operation was modelled by a set of mathematical equations coded into the tool software (Appendix C). The complexity of the models depends on the current needs and available data. Initially, simplistic mass balance process models established on the law of conservation of mass were proposed in the framework. They allowed the product yield and performance of each unit operation to be determined. Such a mass balance approach was employed as it enabled the composition of the different components in the process stream to be determined. The architecture of the software is flexible to accommodate more sophisticated models at various levels of rigour when more data is available.

3.7.5. Representing Uncertainties in Parameter Values

Extend provides the *Input Random Number* block to generate random numbers according to a distribution. To measure the potential risk associated with a given manufacturing option, probability distributions were assigned to the key uncertain factors. The distribution specified the relative frequency with which an event occurred or was likely to occur. Existing plant data and advice from industrial experts were employed to help the identification of appropriate probability distributions. In the case study presented in Chapters 5 and 6, the key uncertainties were found to be in the product fermentation titre, downstream process yields, batch contamination and equipment failure.

3.7.6. Risk Assessment

The Monte Carlo simulation technique was employed to incorporate the randomness inherent in biomanufacture and to evaluate the risks associated with different manufacturing alternatives. The method proposed to handle stochastic parameters was to employ discrete distributions where each possible value of a parameter is given a probability of occurrence. A single trial outcome of the model was determined by selecting a value based on the defined input probabilities. Multiple output scenarios of a model were generated by repeatedly sampling values from the input probability distributions for the uncertain variables. This enabled the resultant frequency distributions of key output parameters and useful statistical summaries to be generated. Details of the specific probability data were supplied where appropriate in the case studies in the relevant chapters.

The decision tree approach to risk analysis was also used to capture the uncertainties in biomanufacture. Such a method selected between several courses of action based on the expected values of each option. The construction of a decision tree started with a decision to be made and branched into possible options. Each branch had an associated probability of occurrence and an outcome. The expected value of an option was calculated by multiplying the outcome with the probability in each path and then adding the weighted values of all the paths for each option. The rule of the decision tree is to select the option which either maximises value or minimises cost.

3.7.7. Data Reporting

The key output parameters from each simulation run are written to Excel and constantly updated during a simulation run. The strengths and well-equipped facilities of Excel for tabulating data, plotting graphs and analysing statistics are then exploited. This facilitates the storage of data for viewing purposes. This is extremely useful for handling the extensive amount of data generated during the Monte Carlo simulations. Each simulation run increments the row number in Excel and logs the output data accordingly in the appropriate cell.

3.7.8. Dynamic Simulation/Animation

In discrete event modelling, discrete entities change state as events occur in the simulation. After completion of a single event, the next event or events are triggered with the simulation clock advancing with each occurrence. Dynamic modelling therefore allows the capability to view the time-based behaviour of the system and makes it possible to track the values of time-dependent parameters. Whenever a simulation is run, the model is animated to enable the user to view the occurrence of events at any instant in time. Animation features enable the visualisation of the flow of items throughout the simulation run and aids in the debugging process for the developer.

3.7.9. Graphical User Interface (GUI)

The graphical representation of the components provides a high degree of user interaction, enables a clear visualisation of various manufacturing stages and offers a comprehensive perspective of the simulation application. The cloned dialog boxes within the manufacturing tasks and resources enable the specification of usage levels with ease. This cloning feature simplifies the communication between the user and the software tool. The 'drag and drop' feature provided by the tool enables rapid development of models.

3.7.10. Profitability Appraisal Measures

There are several financial performance metrics available to measure and compare the profitability of production strategies. The simplest requires a good indication of the total installed cost, the working capital and the operating costs at full production. The most complicated method needs a detailed layout of investments, costs and revenues against time. This section outlines the various appraisal measures. These performance metrics are typical profitability measures used in the literature and industries to provide an assessment of manufacturing options.

3.7.10.1. Fixed capital investment

Capital cost estimates for a plant are based on a factorial method. The fixed capital costs are approximated as a function of the total purchase cost of the equipment in the plant. This method is used as a preliminary and quick approximation when little or no information is available during the early stages of project design. An order-of-magnitude estimate of the fixed capital investment is obtained by multiplying the costs of plant equipment by a factor, termed the Lang factor. The factorial equation of this technique is given by the following equation:

$$C_f = f_L * \sum_{i=1}^n C_{e,i} \quad (3.1)$$

where C_f = fixed capital investment,

C_e = equipment purchase cost,

f_L = Lang factor.

The other cost factors included in the estimation include installation, piping, insulation, instrumentation, electrical auxiliary, storages, utilities, site preparation and contingency costs. The Lang factor depends on the type of process plant. Estimates in the range of 3–5 for chemical plants are recommended (Sinnott, 1993). For biopharmaceutical plants, discussion with industrial experts (e.g. A. Sinclair, Biopharm Services Ltd, Chesham, UK) suggested suitable values in the range of 4–8. These were used as default values in the model.

3.7.10.2. Cost of goods (COG) model

Instead of using built-in cost features in Extend, costing was modelled using existing blocks in the libraries. Both the customised global arrays, *Capital Cost* and *Operating Cost*, were employed to keep track of the running costs incurred during the simulation run. These arrays allow access of data globally in the model by sharing information between blocks in different hierarchies without direct connection. The key cost data entries and outputs were created in Excel and linked to Extend using the *Data Receive* and *Data Send* blocks in the Inter-Process Communication library.

The tool employed a typical cost model, as tabulated in Table 3.5, to calculate the cost of goods (COG). The COG model was derived from cost equations originally developed for conventional chemical engineering facilities (Sinnott, 1993). The factors employed to estimate some of the cost categories (e.g. miscellaneous materials, supervisors, general management, maintenance etc.) were derived from chemical engineering textbooks and were the only data available at the time of implementation. It is recognised that they do not provide definitive values of actual costs (All factors were implemented as fixed values in the simulation tool.). Since the main purpose was to illustrate how such cost categories could be implemented in the tool to compute cost estimates so as to support decision-making in biomanufacturing, the precise values were deemed not to be critical. In addition, supplementary costs associated with the compliance to cGMP in the biopharmaceutical manufacturing industry were considered. The indirect/fixed overheads were derived as a function of the fixed capital investment. The direct costs were computed based on the utilisation of resources such as materials, utilities and staff. The cost of each manufacturing task was computed according to the usage levels of resources.

Table 3.5. Cost of goods model.

Cost category		Equation
Direct	Direct raw materials	$f(\text{utilisation of raw materials})$
	Miscellaneous materials	$0.5 * \text{Direct raw materials}$
	Direct utilities	$f(\text{utilisation of utilities})$
	Operating labour	$f(\text{utilisation of labour})$
	Supervisors	$0.2 * \text{Operating labour}$
	General management	$1.0 * \text{Operating labour}$
Indirect	Maintenance	$0.1 * \text{Capital investment} * \text{Project duration}$
	Local taxes	$0.02 * \text{Capital investment} * \text{Project duration}$
	Insurance	$0.01 * \text{Capital investment} * \text{Project duration}$
	Depreciation	$\text{Capital investment} * \text{Project duration} /$ $\text{Depreciation period}$
	Interest	Interest rate of 10%
	General utilities	$\text{Cost per unit area per year} * \text{Facility size} * \text{Project duration}$

Based on bioprocessing plant data (Personal communication: R. Francis, Protherics Plc., London, UK), it was possible to predict the cost contributions arising from in-process testing and documentation steps (Table 3.6). The costs of the QC/QA and batch documentation labour were calculated as a function of utilisation. The cost of staff per hour was indicated in the resource pool. For each unit operation in the process flowsheet, the requirements of the QC/QA and batch documentation activities were either user-specified or default values employed. The user needed to specify the time taken to complete the QC/QA and batch documentation task. Upon activation of the QC/QA or batch documentation task, the labour cost was computed based on the utilisation rate and cost of the resource. The miscellaneous materials (e.g. equipment and raw materials) associated with the QC/QA and documentation tasks were related to the utilisation of labour. The costs included taking samples, transfer to the QC/QA laboratory, testing, reviewing data and reporting results.

Table 3.6. Cost of goods model for QC/QA and documentation activities.

Cost category	Equation
Quality control/Quality assurance labour	f(utilisation)
Batch documentation labour	f(utilisation)
Miscellaneous materials	10–20% of operating labour time

The cost of goods per gram (COG/g) was presented using a cost category (e.g. indirect costs, labour, material and utilities), a task category (e.g. product-manufacture, equipment-preparation and regulatory-compliance tasks) and a process-specific category (e.g. fermentation, recovery and ancillary steps). This provides the capability of viewing where the bulk of manufacturing costs are distributed for different production strategies. These features of the tool are explored further in the case studies presented in Chapters 5 and 6.

3.7.10.3. Net present value (NPV) model

The economics of a project can be viewed as a series of net cash flows (NCF) throughout the lifetime of a project. To take into account the value of money, these cash flows are discounted to the present time for evaluation (Happel & Jordan, 1975; Allen, 1991). The present value (PV) of a cash flow at the end of a project year t is given by

$$PV_t = \frac{NCF_t}{(1+r)^t} \quad (3.2)$$

where NCF = net cash flow at end of a project year t ,

r = applied discount rate (expressed as a decimal fraction).

The discount factor is chosen to reflect the earning power of money and is equivalent to the current interest rate that the money could earn if invested in a bank. A default value of 9% was assumed for the operating lifetime of the plant (Myers & Howe, 1997).

The NPV of a project is the present value (discounted) of future cash inflows less the present value of the investment and any associated future cash outflows. This factor describes adequately the lifetime profitability of a project by giving appropriate weight to each year's cash flow and indicates whether a project will enhance/diminish the company's financial performance at a particular discount rate. The NPV of a given project is evaluated as follows:

$$NPV = \sum_{t=0}^n PV_t \quad (3.3)$$

where n = complete project life in years.

The typical worksheet for presenting the annual cash flow, discounted cash flow and net present value of a project is tabulated in Table 3.7 (Peters & Timmerhaus, 1991). The PV is normally calculated at a convenient point in time (e.g. start of construction or time of starting the plant into operation). In the model, the PV is calculated at the time of the start of construction i.e. Year 0. The probable life of a project will depend on the type of market and a 5-10 years life is usually achievable. The biomanufacturing plant in the model is assumed to have a construction period of 3 years and a subsequent plant operating life of 10 years.

3.7.10.4. Other profitability metrics

The cost analysis is extended to include other profitability indicators such as the internal rate of return (IRR) and the payback time. The IRR of a project is the discount rate where the NPV at the end of the project is zero. It represents the average return over the lifetime of a project. Typical IRR values for attractive projects are 30% (Peters & Timmerhaus, 1991). The payback time is the period over which the cumulative cash flow is negative. The elapsed time between the start of the project and payback time is the length of time needed to recoup the capital investment.

Table 3.7. A typical worksheet for presenting the annual cash flow, the discounted present value and net present value of a project.

Category	Year (t)				
	0	1	2	...	n
A. Total capital investment					
B. Revenue					
C. Running costs (without depreciation)					
D. Profit (B-C)					
E. Depreciation					
F. Taxable profit (D-E)					
G. Tax (33% of F)					
H. Net cash flow (-A+B-C-G)					
I. Discount factor ($\frac{1}{(1+r)^t}$)					
J. Annual present value (H*I)					
K. Net present value ($\sum_{t=0}^n J_t$)					

3.8. The Implementation Process

3.8.1. Model Testing

The unit operations built in *BioPharmKit* were tested against the original manufacturing units implemented in ReThink. The sequence of manufacturing tasks was connected according to the original case study (Farid, 2001) and the same mass balance data was specified within each unit operation. The model was then simulated to evaluate the composition of the process stream from each unit operation in the tool and such results were compared with that obtained from the previous ReThink model. The cost estimates from the tool were also computed and evaluated in a similar fashion. The outcome of the testing provided confidence in the accuracy of the tool to represent pertinent features in the biomanufacturing environment.

3.8.2. Debugging

The *Generic* and *Discrete Event* libraries provide many blocks that are useful for model debugging. These blocks include the *ReadOut*, *Information*, *Stop* and *Animation Slower/Faster* blocks. The *ReadOut* and *Information* blocks display the value and information about the items respectively. The *Stop* block terminates a simulation run if the value of an item fails a certain threshold value. The animation blocks enable the developer to view the value or items in a block to determine if the simulation is performing as expected. For instance, if the unit operation block show materials passing through, but no material leaves the block, then such information can be used to debug the model. The *Animation Slower* button enables the model to run more slowly in order to debug or critically visualise what the model is doing.

3.9. Conclusions

The development and implementation of a prototype tool to model bioprocesses, including in-process testing and documentation, in a hierarchical task-oriented approach have been presented. Developers can exploit the decomposed framework to add additional features accordingly. Modelling at the higher hierarchical levels provides an overview of the entire process with its key operational and economic parameters. Subsequent details of each higher-level activity can be obtained by breaking down into sub-tasks. The prototype tool developed enables users to capture both process and business knowledge of a manufacturing process in a transparent and organised manner. The modelling of the manufacturing operation provides a transparent representation of the processes. The organised structure acts as a common platform between users and models and enables the systematic evaluation of key performance metrics. The model serves as a bridge for effective communication between different departments within a company such as process development, manufacturing, investment and financial. The simulation results can be applied in assessing the manufacturing options among the decision makers of the company. The next chapter highlights the capabilities of the decision-support tool for industrial applications and outlines the procedure to assemble simulation models of typical manufacturing processes.

CHAPTER 4

Overall Discussion of Tool and its Utility

4.1. Introduction

The previous chapter presented the design and implementation of a prototype tool to model bioprocesses in a hierarchical, task-oriented manner. The proposed framework aims to integrate both business and process perspectives of biopharmaceutical manufacture. Such a computer-aided analysis tool can aid in the facilitation of process design and the appraisal of the relative operational benefits and cost-effectiveness of various manufacturing strategies. The purpose of this chapter is to provide an overall discussion of the decision-support tool, *BioPharmKit*, and illustrate the functionalities of the tool to model cost summation, perform mass balance calculations and simulate resource handling. The benefits of bioprocess simulation are demonstrated by illustrating how plant data can be captured in a flowsheet model to assess process economics.

This chapter is divided as follows. An overview of the decision-support tool and its utility is provided in Section 4.2. The specific capabilities of the prototype tool developed in the previous chapter and the utilisation of such a tool to address key issues in the biomanufacturing industry are discussed. The key input factors required to perform the simulation and the parameter outputs from the prototype tool are also

identified. The steps to assemble a typical model for a specific biopharmaceutical manufacturing process flow are outlined in Section 4.3. Finally, a summary of the conclusions is provided in Section 4.4.

4.2. Tool Overview

4.2.1. Tool Utility

Bioprocess simulation permits a process development team to use available information together with some initial assumptions to begin the creative task of conceptualising process designs and identifying promising options. In this thesis, a prototype tool has been developed to assist the process of decision-making by providing the ability to address key industrial questions and to calculate quantitative metrics for evaluation purposes. Several uses of the simulation tool are highlighted and discussed below:

- *Act as a “blank sheet” for choice of new plant in the design stage*

During the early design stage, there exist a wide range of manufacturing options available to produce a particular drug. The simulation tool was developed to facilitate decision-making during early phase of process development. The user devises a process flowsheet of all unit operations (e.g. fermentation train, purification steps) and supporting ancillary tasks (e.g. cleaning-in-place (CIP), sterilising-in-place (SIP)) based on literature sources or existing industrial practice. Based on these assumptions, the user is able to replicate a flowsheet model of the process. Once the initial model is established, the next step is to examine how the overall process could be improved. The software tool could be employed to analyse alternative processing options from both profitability and feasibility viewpoints.

- *Simulate best usage of plant using existing operating capacity*

To simulate the manufacturing process of an existing operation, it is necessary to model each unit operation in the process. The user is required to specify certain information about each operation in the plant. The unit operations can then be connected to represent the entire process flowsheet. Based on the specifications, the

model will determine the performance of the process. The tool could then be used to expose bottlenecks due to resource constraints. The user can then assess the impact of process modifications so as to find a strategy that minimises production costs, maximises existing plant resources and thus improves the overall viability of the process in the manufacturing plant.

- *Set up a range of case studies to reflect real manufacturing situations*

The tool enables the formulation of models to establish various case studies and to explore the feasibility of different manufacturing strategies through a computer-aided approach. The model could act as a communication channel within the departments of a company such as finance, process development and manufacturing. The simulation results can aid in the appraisal of manufacturing alternatives.

- *Provide a user-friendly interface*

The configuration of the decision-support tool provides a user-friendly interface between the tool platform and the user. The “drag” and “drop” features of the tool enable the user to select the desired block and place it on the relevant workspaces. The dialog boxes within the manufacturing tasks and resources allow users to enter the required values without entering the coding blocks. The graphical user interface of the tool aids in the visualisation of the simulation platform and facilitates the building of a process flowsheet.

- *Investigate scaling-up options*

The scale-up of biological products from clinical trials to commercialisation poses a major obstacle during the development process. The manufacturing challenge for all biopharmaceuticals is to meet the varying demand during the drug development cycle. An increase in product demand may require a simultaneous change in process capacity. Computer-aided design tools could help achieve this goal by formulating models at different scales so as to reduce the time involved in process development.

- *Model ancillary tasks explicitly*

The tool configuration enables ancillary manufacturing tasks (e.g. equipment-preparation and regulatory-compliance tasks) to be modelled explicitly. The task-

oriented approach means that such activities can be modelled separately from the main product-handling tasks in their respective hierarchical workspaces. It is possible to view the cost, duration and yield of each specific manufacturing task. Consequently, the performance metric models (e.g. cost, resource-utilisation profiles) could account for each relevant task category that may otherwise been overlooked.

- *Customise new unit operations*

The modular and extensible framework offers a high degree of flexibility to accommodate improved and more sophisticated models at various levels of details. Such an architecture allows the developer to build and customise new unit operations with ease. Hence, users interested in the feasibility of new technologies may simply model and evaluate these techniques based on the determination of industrial performance metrics. Furthermore, the developer can add features to existing unit operations accordingly to achieve more rigorous and predictive models. Each increase in rigour requires more fundamental data and improves the accuracy of the models while testing against industrial case studies.

- *Incorporate uncertainties and carry out risk analysis*

Existing process modelling and software packages are often deterministic, assuming all processes occur with certainty. In reality the manufacturing environment is subject to numerous random elements inherent in process systems. These risk-related factors may impair the measurement of performance metrics and vitiate project appraisal. In addition to specifying deterministic point entries for parameters, the tool enables variables with probability distributions to be incorporated in process models in order to imitate the underlying randomness in biopharmaceutical manufacture. Combining such risk analysis into the evaluation allows the assessment of potential risks associated with different manufacturing alternatives to be captured and enhances the quality of decision-making within a company.

4.2.2. Simulation Flow

The flow of information during a simulation run is illustrated in Figure 4.1. To develop a process model, the initial task was to formulate the problem and devise a flowsheet. The next step was to gather relevant information for use in the model.

The user then modelled the sequence of unit operations and captured the data in manufacturing recipes. The chemical and physical properties of the process streams were specified in the model. Finally, the operating requirements and conditions were indicated for each unit operation. The task-oriented representation transformed the process knowledge into a graphical model and created a user-friendly interface. After the initial model had been set-up, the simulator can then help to perform bottleneck analysis, cost evaluation and process optimisation. The animated features of the tool aid in the debugging process and enable the visualisation of the occurrence of events during the simulation. The tool generated a report comprising of the capital and operating costs of the plant in an Excel spreadsheet and predicted the process performance, including the compositions and properties of the process streams. The simulation results can be used to determine the profitability and feasibility of the manufacturing option.

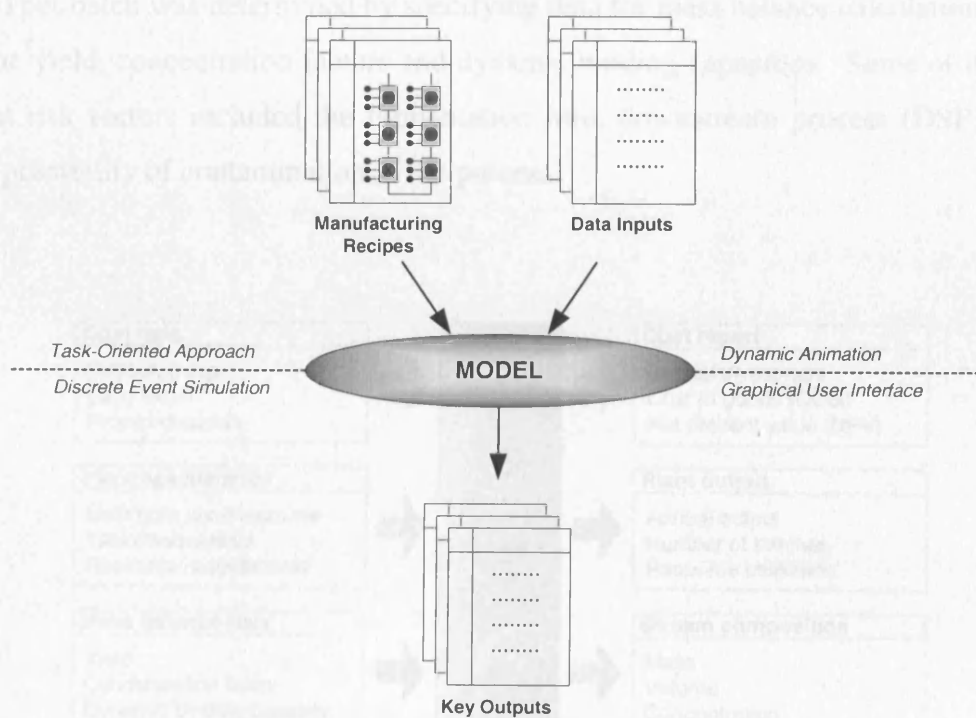


Figure 4.1. Flow of information during a simulation run.

4.2.3. Input/Output Parameters

Figure 4.2 depicts the key input and output parameters of the prototype tool. The tool required that the user either indicated the purchase cost/cost per use of the resources or applied built-in cost models based on cost-estimation factors for bioprocess equipment (Remer & Idrovo, 1991). The Lang factor used to estimate the capital cost and the operating life of the plant was either specified or the default value in the tool was used. Other cost-associated input parameters included the interest rate charged, depreciation period, facility size and cost per unit area of facility. The model required the specification of the maximum availability of all the resources within the biopharmaceutical plant. The series of process operations to manufacture the product, prepare the equipment, test the sample and document the batch were then defined on the relevant hierarchical workspaces. The duration of certain manufacturing tasks were then indicated and the resources required attached to the manufacturing tasks with their usage levels specified. The overall process yield per batch was determined by specifying data for mass balance calculations such as the yield, concentration factors and dynamic binding capacities. Some of the key input risk factors included the fermentation titre, downstream process (DSP) yield and possibility of contamination in the process.

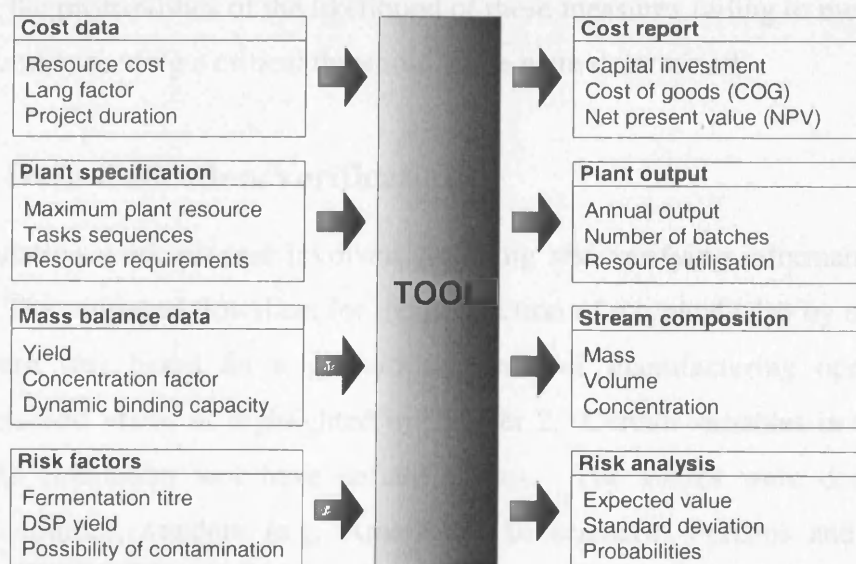


Figure 4.2. Key input and output parameters of the prototype tool.

The total capital investment, which is a crucial factor in estimating the up-front costs needed to start the project, was calculated based on the equipment costs and the Lang factor. The cost of goods (COG) indicated the cost of producing a gram of product taking into account the indirect costs, variable costs and overheads, and was used to gauge the viability of a given manufacturing option. The net present value (NPV) analysis could be used to determine the profitability of a particular manufacturing option over a period of time. The tool computed the annual output generated and the number of batches of product, which can be used to determine whether a particular manufacturing strategy is able to meet a required market demand. The tool also generated the dynamic utilisation profiles of each resource over time. Such utilisation profiles illustrate the demands on resources depending on the manufacturing option and would prompt a company to allocate the appropriate level of resources to carry out any manufacturing task efficiently. Finally, the composition of the components in the process streams from each product-manufacture task was computed. The mass balance inputs were employed in determining the mass and volume of each component in the output streams and other variables such as task processing times and membrane areas. In the risk analysis, the outcomes from the Monte Carlo simulations were used to generate frequency distributions for the performance measures (quantities of Mabs generated, annual COG/g). The expected values and standard deviations of such performance metrics were then derived. In addition, the probabilities of the likelihood of these measures failing to meet a certain demand and exceeding a critical threshold value were determined.

4.2.4. Data Collection/Verification

The simulation work process involved gathering and verifying information for the models. The proposed flowsheet for the production of a typical Mab by mammalian cell culture was based on a generic sequence of manufacturing operations of commercialised Mabs, as highlighted in Chapter 2. Certain variables in the models within the simulation tool have default values. The values were derived from literature sources, vendors (e.g. Amersham Biosciences, PerBio) and industrial experts. The data used in the models were then validated through a series of discussions with experts (e.g. R. Francis from Protherics Plc., London, UK; A. Sinclair from Biopharm Services Ltd., Chesham, UK; B. Fish from Cambridge

Antibody Technology, Cambridge, UK; J. Wayte from Lonza Biologics, Slough, UK and E. Hoglund from Lonza Biologics, Portsmouth, USA) to ensure the values were reasonable. The model data resided in both the Extend database and Excel files. The results of the simulation model were verified by consulting the experts.

4.2.5. Default Input Values

Table 4.1 summarises the default input values used in the model. These values were derived from literature, vendor sources and discussions with industrial experts. As described in the previous chapter, the method for calculating the fixed capital investment was obtained by multiplying the total equipment purchase cost by a factor, traditionally termed the Lang factor. The specific value for the Lang factor depends on the type of process plant being used. For biopharmaceutical engineering facilities, suitable values in the range of 4-8 have been suggested through discussions with industrial experts (e.g. A. Sinclair, Biopharm Services Ltd, Chesham, UK). The Lang factor was assumed to have a base value of 6. The default value for the annual facility cost of general utilities per unit floor area was assumed to be \$300/m² (M. Sawyer, Lonza Biologics, Portsmouth, US).

Table 4.1. A summary of default input values used in the model.

Category	Assumptions	Input value
Economic data	Lang factor	6
	Facility cost per unit area	\$300/m ²
	Interest rate	10%
	Depreciation	10% of fixed capital investment
	Cost-estimation factors	0.3–0.7
Time factors	Project duration	10 years
	Plant operating time	48 weeks/year, 7 days/week, 24 hours/day
	QC/QA & documentation	Operating hours, i.e. ~8 hours a day
	Test a batch manually	0.5–1 day
	Document a batch manually	15%–20% of operating time
Plant capacity	Operator unit	10
	QC/QA & documentation unit	40% of total plant operating force

The interest charges relating to capital expenditure should be taken into account in the economic evaluation of a project. The interest rate on the investment was taken as 10% (Personal communication: A. Sinclair, Biopharm Services Ltd, Chesham, UK). The operating life of a biotech facility is usually taken as 10 years, which gives a depreciation rate of 10% per annum.

A key stage in calculating the capital investment for a production plant is determining the total equipment purchase cost. An estimate of the cost of a new equipment can be obtained from a known cost for that type of equipment and the ratio of their capacities raised to an index value. The cost is related to the capacity by the following equation:

$$C_2 = C_1 \left(\frac{Q_2}{Q_1} \right)^n \quad (4.1)$$

where n = index value,

C_2 = cost of equipment with capacity Q_2 ,

C_1 = cost of equipment with capacity Q_1 .

Process engineers often use the well-known six-tenths rule, where the value of the index, n , is traditionally taken as 0.6 (Sinnott, 1993). However, Remer & Idrovo (1991) warned against the blind use of such exponent value and indicated exponential scaling factors for 58 different types of sizes of bioprocess equipment. This exponential method was used to provide a quick estimate of the investment likely to be required. The costs for known resources are tabulated in Table 4.2, indicating that purchase costs were specified for equipment and unit costs for the remaining resource categories. The cost inputs were determined from industrial experts and vendor sources (e.g. Biopharm Services Ltd, Protherics Plc., Millipore, Pharmacia, PerBio Science). The costs of related resources in subsequent case studies were calculated based on cost-estimation factors (Remer & Idrovo, 1991). Some of the key factors for the mass balance calculations are indicated in Table 4.3. The default values for the process data was estimated using information supplied by vendors and discussions with industrialists.

Table 4.2. Base cost data for the resources.

Resource	Description	Cost (\$)
Equipment	Shake flasks (10), V = 0.5L	300
	Seed fermenter, V = 20L	80,000
	Production fermenter, V = 1,000L	525,000
	Centrifuge	250,000
	Affinity chromatography rig & column, D = 60cm	280,000
	Virus retrovirus inactivation tank	45,000
	Diafiltration rig	75,000
	Ion exchange chromatography rig & column, D = 60cm	280,000
	Nanofiltration rig	75,000
	Gel filtration chromatography rig & column, D = 60cm	280,000
	Dead-end filtration rig	75,000
	Vessel/Holding tank, V = 1,000L	37,500
	Container used to hold a 200L disposable bag	1,000
	CIP vessel	120,000
Equipment-related materials	Membrane cassettes	1936/m ²
	50nm virus reduction filter (0.05m ²)	1,000/unit
	0.22μ cartridge filter (0.05m ²)	75/unit
	Protein A matrix	7,500/L
	Ion exchange matrix	420/L
	Gel filtration matrix	330/L
	200L disposable bag	300
Chemicals	Media	20
	Buffer	5
Labour	Operator	25/h
	QC/QA staff	40/h
Utilities	WFI	1/L
	Steam	0.05/kg
	Cooling water	0.001/L

Table 4.3. Key mass balance inputs.

Task	Input factor	Input value
Fermentation	Mass stoichiometry coefficients	Media: -1.5; Cells: 1; IgG: 0.5; Others: 0
	Limiting substrate	Media
	Reference component	IgG
	Fermentation titre	0.2g/L
Affinity chromatography	Binding capacity, g/L	20
	Linear flowrate, cm/h	Load: 150; Wash: 150; Elution: 75
	No of column volumes	Wash: 10; Elution: 5
	Product-stream-column-volumes	4
	Yield fraction	Media: 0; Cells: 0; IgG: 0.9; Others: 0
Ultrafiltration	Average flux, L/m ² /h	20
	Concentration factor	10
	No of cycles	1
	Rejection coefficients	Media: 0; Cells: 1; IgG: 1; Others: 0
Diafiltration	Average flux, L/m ² /h	20
	No of diafiltration volumes	4
	No of cycles	1
	Rejection coefficients	Media: 0; Cells: 1; IgG: 0.99; Others: 0
Ion exchange chromatography	Binding capacity, g/L	20
	Linear flowrate, cm/h	Load: 150; Wash: 150; Elution: 100
	No of column volumes	Wash: 10; Elution: 10
	Product-stream-column-volumes	2
	Yield fraction	Media: 0; Cells: 0; IgG: 0.96; Others: 0
Gel filtration chromatography	Volumetric capacity	10% column volume
	Linear flowrate, cm/h	Load: 12.5; Elution: 12.5
	No of column volumes	Elution: 1
	Product-stream-column-volumes	0.3
	Yield fraction	Media: 0; Cells: 0; IgG: 0.95; Others: 0

4.3. Using the Prototype Tool

The following procedure takes the user through the steps of simulating an upstream process (USP) operation. Each of the resource pools was first declared in their respective workspaces. The series of product-manufacture tasks were connected in the relevant task recipe. The required resources were then linked to the input connectors of these tasks. The same procedure applied to those of the supporting manufacture tasks (e.g. CIP, SIP). The key input variables were entered prior to the start of the simulation.

4.3.1. Declaration of Resource Pools

The manufacturing resources were cloned from their respective resource category and positioned on the relevant workspaces. The resource element comprised both the resource pool manager and the resource. Figure 4.3 illustrates the placement of the resource managers in the resource pool declaration windows.

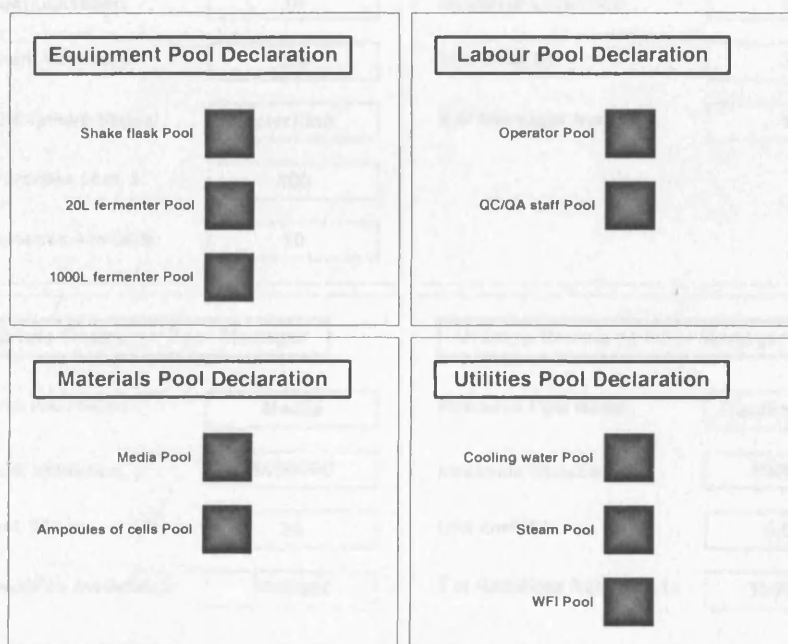


Figure 4.3. Declaration of resource pools. Each of the resources was cloned from the main category and positioned on their respective workspaces.

The resource itself was attached to the manufacturing task. For example, the *shake flask* resource manager was duplicated from the equipment library and placed on the equipment pool declaration window. The USP operation consisted of the equipment resources (i.e. shake flasks, seed and production fermenter), labour resources (i.e. operator and QC/QA staff), material resources (i.e. media and cells) and utilities resources (i.e. cooling water, steam and WFI). Each of the input entries in the resource pool was then declared. Figure 4.4 shows the user inputs in solid boxes. The user specified the resource pool name, maximum utilisation and cost. For the equipment resource, the initial equipment status and equipment size were entered. The dashed boxes indicated output values; the “number of resources available” was constantly updated during a simulation run to indicate the current available resources in the pool while the total purchase cost for the equipment resource was determined at the start of the simulation.

Equipment Resource Pool Manager		Labour Resource Pool Manager	
Resource Pool Name:	Shake flasks	Resource Pool Name:	Operator
Maximum Utilisation:	10	Maximum Utilisation:	10
Equipment Volume, L:	0.5	Unit Cost, \$/h:	25
Initial Equipment Status:	sterilise	# of Resources Available:	10
Total Purchase cost, \$:	300		
# of Resources Available:	10		

Materials Resource Pool Manager		Utilities Resource Pool Manager	
Resource Pool Name:	Media	Resource Pool Name:	Cooling water
Maximum Utilisation, L:	1000000	Maximum Utilisation, L:	1000000
Unit cost, \$/L:	20	Unit cost, \$/L:	0.001
# of Resources Available, L:	1000000	# of Resources Available, L:	1000000

Figure 4.4. Examples of resource pool managers. The solid boxes represent user inputs while the dashed boxes indicate output values.

4.3.2. Set-up of Manufacturing Tasks Recipes

After specifying the manufacturing resources in the plant, the series of manufacturing tasks were defined. Figure 4.5 illustrates the detailed graphical representation of a typical USP generated by cloning the customised manufacturing task blocks from libraries and then positioning them on the respective recipes. In the *process-flowsheet* workspace, the sequence of product-manufacture tasks was established. The product-manufacture steps of inoculum grow-up and seed fermentation to the production fermentation were linked in series with the resources attached to the input connectors. The top input connector from the *inoculum grow-up* task was linked to the output connector of the *Start* block. The *20L* and *1,000L fermentation* processes were then linked accordingly, with the *End* block being the last block. The necessary resources were then connected to each of these tasks. The user specified the utilisation level of each of these resources or the default values were employed. The current task was activated by the process stream item flowing into the block from the prior step. In the *equipment-cleaning-in-place* window, the *equipment* resource was connected to the top connector of the preparation task. The relevant resources were then connected. The preparation tasks ended with an *Exit* Block. This block displayed the run number for the particular task. The same procedure applied to the *equipment-sterilising-in-place* and *regulatory-compliance* recipes. Figure 4.5 also illustrates part of the process flowsheet in action. The *20L fermentation* process was animated during the simulation, allowing the user to view what was happening at a particular time of the simulation.

4.3.3. Input Parameters

The cost-associated input parameters (e.g. Lang factor, project duration, facility size) for the model were entered. The factors for mass balance calculations were input to determine the characteristics (i.e. mass, volume) of the process streams from each process step. The key mass balance data included the fermentation titres, process yields, and dynamic binding capacities. These statistics were either selected from the list of available distributions in the tool or user-defined distributions with known probabilities were used. Finally, the simulation set-up parameters, including the simulation time (in days) and the number of runs, were then specified.

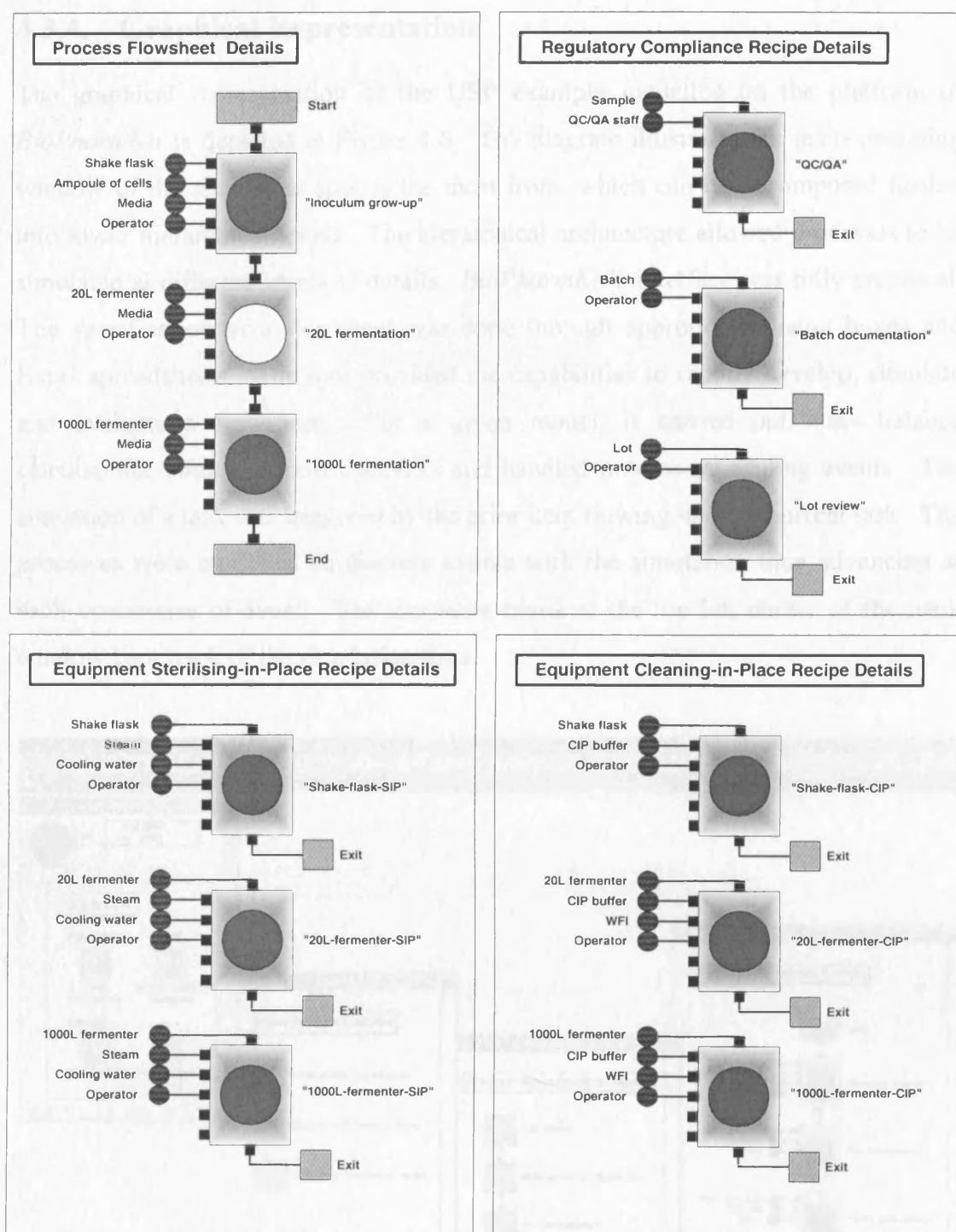


Figure 4.5. An example of a typical USP defined in the recipes, with the required resources attached to the tasks. The diagram depicts an instance of the discrete simulation process in action, with an active *20L fermentation* task being animated during the simulation cycle. The resources (e.g. equipment, material, labour, utilities) in the model were generated by cloning the customised blocks from the libraries and then connected.

4.3.4. Graphical Representation

The graphical representation of the USP example modelled on the platform of *BioPharmKit* is depicted in Figure 4.6. The diagram illustrates the main operating window of the simulation tool at the most front, which can be decomposed further into lower hierarchical levels. The hierarchical architecture allowed processes to be simulated at different levels of details. *BioPharmKit*'s interface was fully graphical. The specification of a flowsheet was done through appropriate dialog boxes and Excel spreadsheets. The tool provided the capabilities to rapidly develop, simulate and evaluate a flowsheet. For a given model, it carried out mass balance calculations, computed cost estimates and handled process-scheduling events. The activation of a task was triggered by the prior item flowing into the current task. The processes were modelled as discrete events with the simulation time advancing at each occurrence of event. The *Executive* block at the top left corner of the main window kept track of the simulation time.

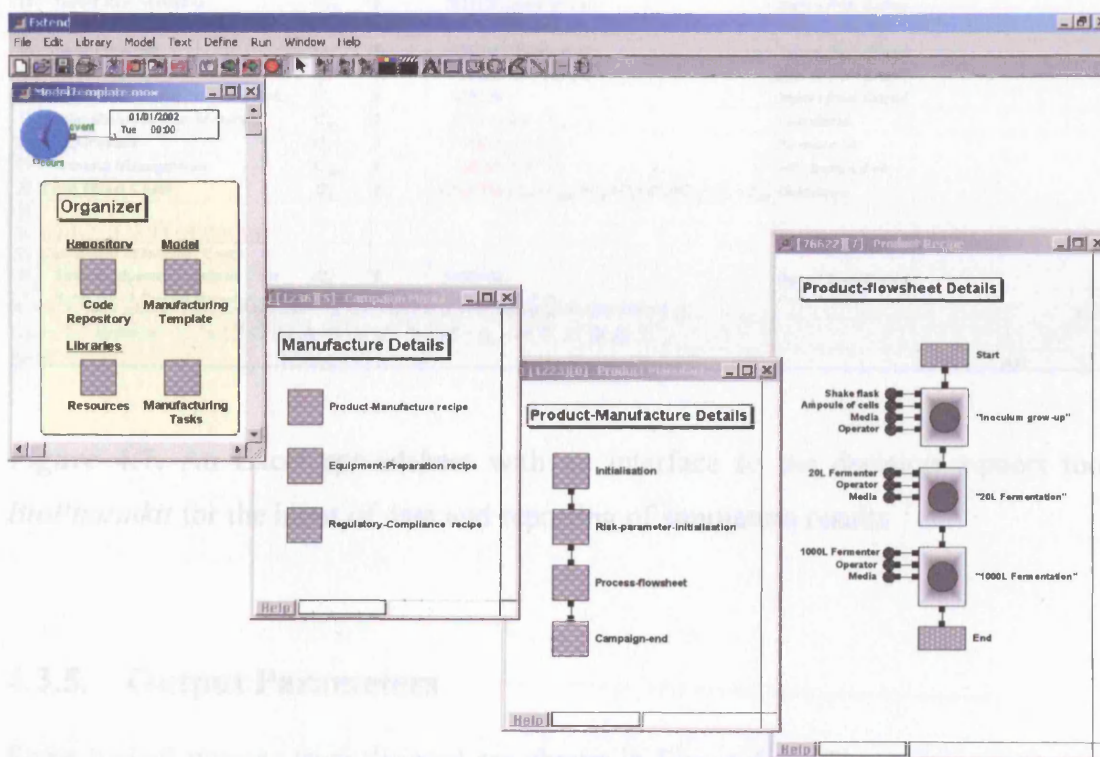


Figure 4.6. Graphical representation of the USP example modelled on the platform of the simulation tool *BioPharmKit*.

The tool provided an interface with Excel for the input of data and reporting of simulation results from the models (Figure 4.7). The user entered the input data prior to the start of a simulation run. The output entries were computed and automatically updated during the simulation. The transparent platform of Excel provided the user with the flexibility of modifying them.

	Notation	Units	Value	Equation	Comments
DATA BANK FOR DECISIONAL SUPPORT TOOL - BIOPHARMKIT					
Users are required to input all values in red!					
KEY INPUT PARAMETERS					
Lang Factor	f_L	-	6		User Input
Project Duration	t_d	yr	1		User Input
Depreciation Period	t_p	yr	10		User Input
Facility Size	A	m^2	1000		User Input
Cost Per Unit Area	C_A	$\$/m^2$	300		User Input
DIRECT COST OF GOODS					
Calculation of Direct Costs -					
Direct Raw Materials	C_{rm}	\$	7635655	$f_{utilisation}$	Import from Extend
Miscellaneous Materials	C_{mm}	\$	3817828	$0.5 * C_{rm}$	Gloves, coats etc.
Direct Utilities	C_u	\$	330649	$f_{utilisation}$	Import from Extend
Operating Labour	C_t	\$	169090	$f_{utilisation}$	Import from Extend
In-process validation/Assay staff	C_q	\$	203275		Import from Extend
Miscellaneous Raw Materials	C_m	\$	203275	$0.2 * C_q$	Calculated
Supervisors	C_s	\$	33818	$0.2 * C_t$	Foreman etc.
General Management	C_{gm}	\$	169090	$1.0 * C_t$	HR, financial etc.
Total Direct Costs	C_D	\$	12562680	$C_{rm} + C_{mm} + C_u + C_t + C_q + C_m + C_s + C_{gm}$	Calculated
INDIRECT COST OF GOODS					
Calculation of Indirect Costs -					
Total Equipment Purchase Cost	C_e	\$	2302965		Import from Extend

Figure 4.7. An Excel spreadsheet with an interface to the decision-support tool *BioPharmkit* for the input of data and reporting of simulation results.

4.3.5. Output Parameters

Some typical outputs from the tool are shown in Figure 4.8. The cost outputs were updated constantly in Extend and Excel during a simulation run. Such data could then be manipulated in Excel to plot out useful graphs for comparing and evaluating the manufacturing option. The utilisation curves in Figure 4.8 suggest the current utilisation level of media and operator over a period of time. These utilisation

profiles could be used to compare the demand on resources for different manufacturing strategies and to allocate the appropriate number of resources to carry out a manufacturing task efficiently. The operator plot indicates that typically, a maximum of 2 operators were employed during the majority of the simulation time. Such figures could be used to optimise resource utilisation to improve productivity and throughput.

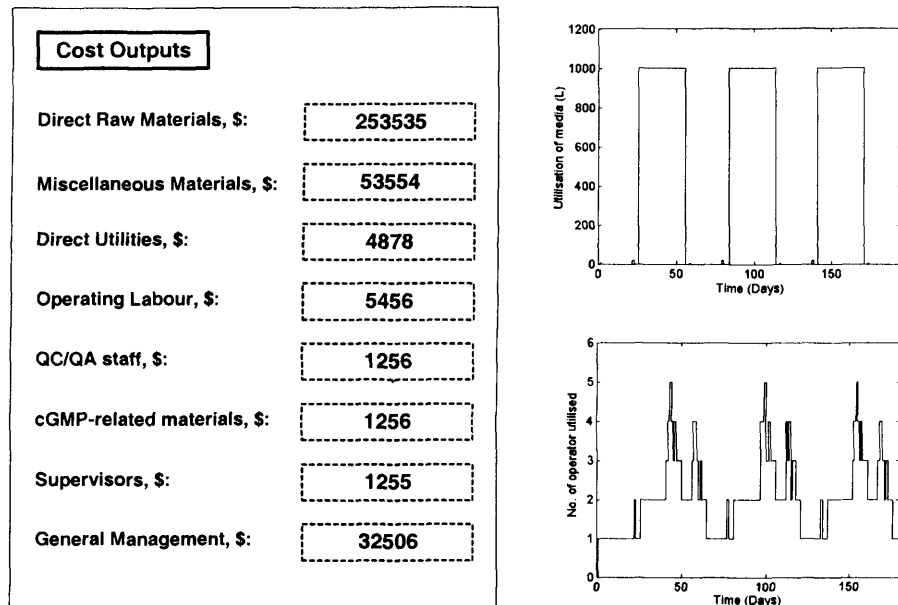


Figure 4.8. Key output parameters. The diagram illustrates cost outputs in different categories (e.g. direct materials, raw materials, utilities etc.). The utilisation curves depict the usage level of resources at a particular time.

4.4. Key Influencing Variables

The decision-making process is influenced by various input variables to the model. In this thesis, a range of typical key variables which each have an impact on the output parameters (i.e. cost of goods, annual throughput) were selected for study. These included process factors such as fermentation yields, titres, turnaround time, possibilities of contamination and equipment failure. Other factors included the Lang factor and cost of resources. Other input factors that impact the performance metrics of manufacturing alternatives were excluded following discussions with

industrial experts. As a result, the key input factors considered were typical variables influencing the process and business aspects of biomanufacturing and were selected to demonstrate the functionalities of the decision-support tool.

4.5. Conclusions

This chapter has provided an overall discussion of the prototype tool. Its utility in the biomanufacturing industry has been highlighted. Such a bioprocess simulator is a potentially valuable and powerful tool to aid the understanding of the complexities of a manufacturing operation and assess the process performance with speed and ease. The input parameters required for the successful formulation of the models and the key outputs generated by the tool have been identified. An example of the simulation of an USP has been used to demonstrate the application of such a software tool to model bioprocesses. The procedure to assemble the model of the USP operation has been presented. This effectively permits the manufacturing operation to be evaluated in terms of key process performance metrics such as the running costs and resource utilisation profiles. The next two chapters use this formula and present two industrial-related case studies that examine alternative processing routes with respect to the technical and financial aspects of biopharmaceutical manufacture. The studies have been configured so as to reflect real manufacturing situations and are typical of current issues facing the biomanufacturing industry.

CHAPTER 5

Application of BioPharmkit for Strategic Biopharmaceutical Manufacture

5.1. Introduction

During the early stage of process development, biopharmaceutical companies typically have a range of manufacturing options by which to produce a particular drug. The decision to operate a given manufacturing option is largely determined by financial budgets, plant capacity and market demand but is further complicated by technical and market uncertainties occurring in the biomanufacturing environment. This chapter explores the utility of the simulation tool, *BioPharmKit*, developed and implemented in Chapter 3, to provide cost estimates, perform mass balance calculations, simulate resource handling, model regulatory-compliance activities, and evaluate potential risks so as to predict the feasibility of different manufacturing options for the production of a biopharmaceutical drug for commercial use. A case study, based upon the assessment of pooling strategies in the perfusion culture of mammalian cells to deliver a therapeutic protein for commercial use, is used to demonstrate the functionalities of the tool. The study aims to investigate the most appropriate pooling strategy given a particular set of operating constraints and goals in a biopharmaceutical-manufacturing environment.

The biomanufacturing environment is often subject to numerous technical and market uncertainties. The Monte Carlo simulation technique is commonly used to study the effect of such uncertainties on the performance measure of stochastic processes. The probability functions of uncertain factors are specified to capture the randomness inherent in bioprocesses. During a single trial of the model, the outcome is determined by randomly selecting a value from the defined input probabilities for each uncertain variable. The expected outcomes of stochastic processes are obtained by running many iterations of the model process and averaging the outcomes together. Such a technique enables a range of possible outcomes to be generated and tabulates useful statistics such as the likelihood of achieving or exceeding a threshold value. The use of such risk analysis method for decision-making is investigated in this chapter.

The remainder of this chapter is structured as follows. In Section 5.2, an overview of the case study, which assesses pooling strategies in perfusion culture, is described and the procedure carried out to simulate the case study model is also outlined. A deterministic analysis of the problem is provided in Section 5.3. In Section 5.4, a sensitivity test is carried out to identify variables influencing key output parameters. A Monte Carlo simulation technique is then applied in Section 5.5 to incorporate the randomness inherent in biomanufacture so as to investigate the effect of variability of process parameters. The case study is extended to explore the impact of different scenarios on the key performance measures in Section 5.6. Finally, a summary is provided in Section 5.7.

5.2. Case Study Background

5.2.1. Case Study Set-up

To evaluate the functionalities of the prototype tool, the production of a therapeutic Mab for commercial use using perfusion culture was considered. The case study was based on a biopharmaceutical company interested in producing an antibody using perfusion culture of mammalian cells. During early phase of process development, they wanted to investigate how often to pool harvested liquor during perfusion culture and hence determine the pooling interval that minimises costs and uses

resources more effectively at commercial scale. There was little information available about how to select the most efficient pooling interval; hence the tool was applied in the case study to explore the impact of pooling intervals on the feasibility of the different manufacturing alternatives. The company considered 3 pooling strategies at 1-, 15- and 30-day pooling intervals. The downstream process (DSP) equipment was sized according to the pooling interval, i.e. a short pooling interval (e.g. 1-day) will require smaller DSP equipment than a high pooling interval (e.g. 30-day). The antibody product was assumed to be stable. As discussed in Chapter 2, the advantages of pooling more frequently include better utilisation of downstream equipment and lower investment costs. However, this strategy suffers from increased equipment-preparation and regulatory costs due to more frequent processing. A less frequent pooling option possesses higher capital costs due to the need for larger DSP and ancillary equipment but has reduced ancillary equipment-preparation and regulatory-compliance steps.

Table 5.1 specifies the key assumptions of the case study validated through discussions with industrial experts (A. Sinclair, Biopharm Services Ltd, Chesham, UK; B. Fish, Cambridge Antibody Technology, Cambridge, UK; R. Francis, Protherics Plc., London, UK). Such advice from industrialists was critical in identifying the logistics of processes within a biopharmaceutical plant and selecting sensible inputs. However, the main concern was to demonstrate the functionalities of the support tool through a case study so as to prove its use as a general approach towards process design and development, and ultimately as an aid in the process of decision-making in the biomanufacturing industry. Hence, the case study does not provide definitive answers but is a representative of such process assessment. In this case study, it was assumed that the commercial facility/plant would have a single production train to manufacture the antibody drug (This scenario might not reflect real manufacturing situations but was used here to demonstrate the use of the tool to investigate different manufacturing options.). The size of the plant would vary according to the pooling interval. A typical product titre of 200mg/L was assumed and the bioreactor was operated at a perfusion rate of 1 reactor volume per day. The collection of the product-enriched liquor commenced after 5 days when a constant growth state was achieved. In each strategy, the unit operations in the downstream recovery steps generated the same overall product yield. All three options were

designed to produce an annual product output of 20kg, which is a typical industrial figure, to meet the anticipated demand. This case study was focused on the operational aspects of the process; hence, the construction and start-up time for the plant was not incorporated in the model. The output parameters of interest were the annual cost of goods per gram (COG/g) and the demand on resources. The feasibility of each of the pooling options was then evaluated and ranked according to these performance measures.

Table 5.1. Key process assumptions in the case study.

Assumptions	Input value
Length of single perfusion run	35 days
Pooling intervals	1, 15, 30 days
Fermentation titre	200mg/L
Size of production bioreactor	1,000L
Seed bioreactor train	20L; 100L
No. of days to achieve constant growth	5 days
No. of production trains	1
Perfusion rate	1 reactor volume/day
Downstream process yield	55%
Product stable	Yes
Annual operating time	48 weeks/year, 24 hours/day

An overview of the process used in the case study for the production of a therapeutic Mab using mammalian cell culture is illustrated in Figure 5.1. The upstream processing commenced with the use of a single ampoule to inoculate a few litres of media. After growth, this lab-scale culture was used to inoculate the media in a small bioreactor. This seed culture was used to inoculate another seed bioreactor and the subsequent culture was then used to inoculate the production-scale bioreactor. During the perfusion culture, cells were retained in the bioreactor and liquor containing the product was collected. The harvested liquor was loaded onto the affinity column, eluted as purified product and stored in disposable bags. The eluate from successive chromatography runs was then pooled for further purification; this was defined as a lot. Viral clearance was followed by further concentration of the broth. The concentrated filtrate was then passed through an ion exchange column.

Further virus reduction by nanofiltration and concentration of the process stream then took place prior to gel filtration as a polishing step. A final filtration step was used, yielding an essentially pure antibody preparation. At the end of the downstream steps, there was a lot review to determine product acceptance/rejection. The key equipment and material requirements for the main product-manufacture tasks in the process flow are tabulated in Table 5.2. For each pooling interval, the size of the equipment used in the upstream stages (i.e. cell culture/fermentation) and affinity chromatography was the same. The sizes of the DSP equipment (boxed in Figure 5.1) for each pooling option are given in Table 5.3.

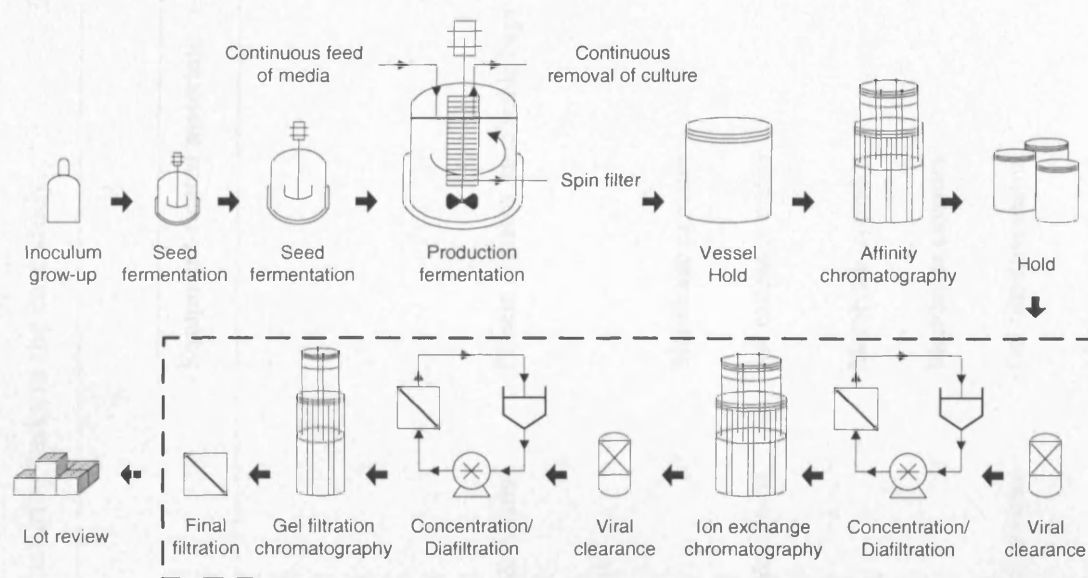


Figure 5.1. Process diagram of the case study: Production of a therapeutic Mab from mammalian cell culture using perfusion culture. The equipment sizes for the DSP steps (shown in the box) varied accordingly to the pooling interval.

In addition to these product-manufacture steps, the model also considered ancillary operations such as equipment-preparation steps (e.g. cleaning-in-place (CIP) and sterilising-in-place (SIP)) and regulatory-compliance activities (e.g. quality control (QC), quality assurance (QA) and batch documentation) running in parallel with the main production train. The requirements of these ancillary operations for each product-manufacture task are shown in Table 5.4. In this case study, for simplicity, media and buffer were assumed to arrive pre-made and pre-sterilised in disposable bags and hence no media and buffer preparation steps were needed.

Table 5.2. Key resource requirements for the manufacturing tasks in the case study.

Manufacturing task	Equipment	Materials	
		Equipment-related materials	Chemicals-&-Biochemicals
Inoculum grow-up	Shake flasks	–	Media, Ampoule of cells
Seed fermentation	Seed fermenter	–	Media
Production fermentation	Production fermenter	–	Media
Affinity chromatography	Chromatography rig & column	Protein A matrix, disposable bags	Protein A wash & equilibration buffer, Protein A elution buffer
Chemical retrovirus inactivation	Virus inactivation tank	–	Low pH solution
Concentration/Buffer exchange 1	Diafiltration rig	Membrane cassettes	Diafiltration buffer
Ion exchange chromatography	Chromatography rig & column	Ion exchange matrix	Ion exchange wash & equilibration buffer, Ion exchange elution buffer
Virus reduction nanofiltration	Nanofiltration rig	Membrane capsules	–
Concentration/Buffer exchange 2	Diafiltration rig	Membrane cassettes	Diafiltration buffer
Gel filtration chromatography	Chromatography rig & column	Gel filtration matrix	Gel elution buffer
Final filtration	Dead-end filtration rig	Membrane capsules	–

Table 5.3. Equipment sizes for the downstream process steps*.

Manufacturing task	Resource pool name	Sizes		
		1-day	15-day	30-day
Chemical retrovirus inactivation	Virus inactivation tank	V = 200L	V = 1,500L	V = 3,000L
Concentration/Buffer exchange 1	Diafiltration pellicon cassettes	A = 2m ²	A = 20m ²	A = 30m ²
Ion exchange chromatography	Ion exchange chromatography rig & column	V = 9L; H = 15cm; D = calculated	V = 45L; H = 15cm; D = calculated	V = 65L; H = 15cm; D = calculated
Virus reduction nanofiltration	Virus reduction filter	A = 0.3m ²	A = 3m ²	A = 4.5m ²
Concentration/Buffer exchange 2	Diafiltration pellicon cassettes	A = 3m ²	A = 30m ²	A = 45m ²
Gel filtration chromatography	Gel filtration chromatography rig & column	V = 18L; H = 50cm; D = calculated	V = 80L; H = 50cm; D = calculated	V = 120L; H = 50cm; D = calculated
Final filtration	Cartridge filter	A = 0.2m ²	A = 2m ²	A = 3m ²

* Verified through discussions with industrial experts (e.g. R. Francis, Protherics Plc., London, UK; J. Savery & R. Spurling, Biopharm Services Ltd., Chesham, UK).

Table 5.4. Specifications for the requirements of ancillary operations.

Manufacturing task	Ancillary operations						
	CIP	SIP	Equilibration	Regeneration	Re-equilibration	QC/QA	Documentation
Inoculum grow-up	✓	✓	x	x	x	✓	✓
Seed fermentation	✓	✓	x	x	x	✓	✓
Production fermentation	✓	✓	x	x	x	✓	✓
Affinity chromatography	✓	x	✓	✓	✓	✓	✓
Chemical retrovirus inactivation	✓	✓	x	x	x	✓	✓
Concentration/Buffer exchange 1	✓	x	x	x	x	✓	✓
Ion exchange chromatography	✓	x	✓	✓	✓	✓	✓
Virus reduction nanofiltration	✓	x	x	x	x	✓	✓
Concentration/Buffer exchange 2	✓	x	x	x	x	✓	✓
Gel filtration chromatography	✓	x	✓	x	✓	✓	✓
Final filtration	✓	✓	x	x	x	✓	✓

5.2.2. Methods

Declaration of resource pools

The resource pools for each of the resources (e.g. equipment, materials, labour, utilities) were cloned from their respective libraries and placed on the manufacturing template accordingly. For instance, the resource *fermenter* was created from the renewable equipment category while the resource *steam* was created from the non-renewable utilities category. The user specified the purchase cost/cost per use of the resources or built-in cost models based on cost-estimation factors for biopharmaceutical process equipment were used. The base cost input data for the resources in the manufacturing plant are given in Chapter 4. Additional inputs of the attributes of equipment included their sizes (e.g. “equipment volume”, “equipment height”, “equipment diameter”) and their initial status (e.g. ‘dirty’, ‘clean’, ‘sterile’, ‘equilibrated’).

Equipment-related materials such as membrane cassettes, capsules and chromatography matrices were assumed to be re-usable for a given number of cycles. The cycle limit of the renewable equipment-related materials was set to 100. For the membrane filtration materials, the working-area was either specified by the user or determined by process and mass balance data. The *chemicals-and-biochemicals* resources were created from the non-renewable material resource category. For simplification, the culture media was defined as one element without breaking down into the individual components such as glucose, water etc. Further attribute inputs (e.g. “mass”, “volume”, “density”, “concentration”, “physical state” and “virus titre”) were required for the chemicals resources (e.g. wash, equilibration and elution buffer) to feature their characteristics.

Definition of main/ancillary tasks sequences

The next step was to define the sequences for the product-manufacture steps and the ancillary tasks of equipment-preparation, in-process testing and batch documentation. An example of the graphical representation of the *product-manufacture* recipe for the perfusion case is illustrated in Figure 5.2. The series of unit operations from fermentation to the final filtration were linked to represent the whole process within the manufacturing environment.

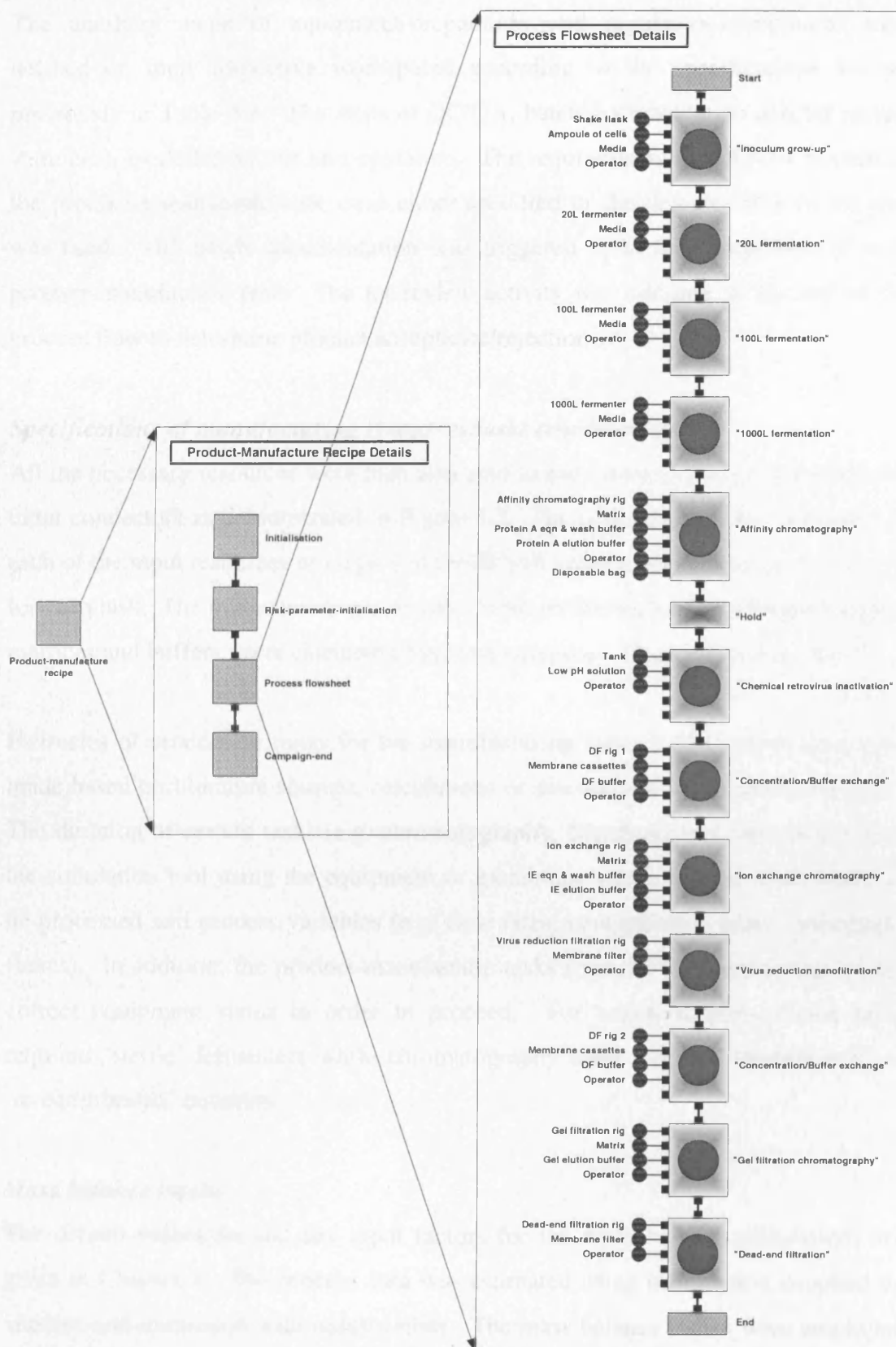


Figure 5.2. Graphical representation of the *product-manufacture* recipe for the perfusion case.

The ancillary steps of equipment-preparation and regulatory-compliance were defined on their respective workspaces according to the specifications set out previously in Table 5.4. The steps of QC/QA, batch documentation and lot review were each modelled as one unit operation. The requirements for QC/QA in each of the product-manufacture task were either specified or the default value in the tool was used. The batch documentation was triggered upon the completion of each product-manufacture task. The lot review activity was initiated at the end of the process flow to determine product acceptance/rejection.

Specifications of manufacturing resources/tasks requirements

All the necessary resources were then allocated to each manufacturing task using the input connectors as demonstrated in Figure 5.2. The user specified the utilisation of each of the input resources or employed the default value to set the resource demands for each task. The utilisation levels of some input resources, such as chromatography matrices and buffers, were calculated based on equipment sizes and process data.

Estimates of processing times for the manufacturing tasks (e.g. fermentation) were made based on literature sources, calculations or discussions with industrial experts. The duration of certain tasks (e.g. chromatography, filtration) was computed within the simulation tool using the equipment or membrane sizes, volume of materials to be processed and process variables (e.g. flow rates, concentration factor, membrane fluxes). In addition, the product-manufacture tasks required the specification of the correct equipment status in order to proceed. For example, fermentation tasks required ‘sterile’ fermenters while chromatography tasks required ‘equilibrated’ or ‘re-equilibrated’ columns.

Mass balance inputs

The default values for the key input factors for the mass balance calculations are given in Chapter 4. The process data was estimated using information supplied by vendors and discussion with industrialists. The mass balance inputs were employed in determining the mass and volume of each component in the output streams, certain task processing times and membrane areas. In each pooling scenario, the mass balance models for the unit operations in the product-manufacture tasks generated the same overall product yield.

Initialisation

The emergence of the process stream of the previous task was used to trigger the next task. The activation of the unit operations was further controlled by the equipment status and the availability of all required resources. In this case study, a new batch was initiated after completion of the production fermentation step. This assumption was applied in this particular case study to illustrate the functionalities of the tool and do not reflect real manufacturing situations. However, the user could define the commencement of a new batch to be triggered by any upstream process step. The ancillary tasks were activated using the *Throw* and *Catch* blocks as described in Chapter 3.

Having set up the flowsheet model and gathered the relevant inputs, the software tool was used to run the different scenarios independently and to compare the results. The simulation outputs were again verified with industrial experts to validate the findings. A deterministic study of perfusion culture was initially carried out using single-point measures. To take into account the inherent variability of process factors, key uncertainties in the system were identified and subjected to a degree of change so as to investigate the impact on output parameters. The uncertainties considered in this case study were the fermentation titre, process yield, turnaround time, Lang factor, media cost, WFI cost and operator cost. In reality, there exist other random factors in bioprocesses and the variables chosen in this study were used mainly to investigate the impact of uncertainties on the stability of the base case.

A Monte Carlo analysis was then performed to help minimise the risk of incorrect conclusions based on the variability in the process parameters. The key uncertain factors used for the Monte Carlo simulation were derived from the sensitivity analysis. The factors investigated were those variables that had the most impact on the output parameters. The input probability distributions of these uncertain variables were specified to represent the inherent randomness in such parameters. The outcome for a single trial of the model was determined during a simulation where values were sampled from the input probability functions of each uncertain variable. A range of possible outcomes was obtained by running many iterations of the model. By monitoring the running averages of the output parameters, the number of simulation runs was repeated until convergence was reached. The frequency

distributions of performance measures were then plotted and useful statistical summaries (i.e. expected values, standard deviations and likelihood of achieving or exceeding a criterion value) tabulated. Some possible scenarios were identified to explore the impact of such implications on key performance metrics. The Monte Carlo simulation technique was employed in each scenario and the manufacturing options were ranked according to the statistics derived from such an approach.

5.3. Initial Deterministic Analysis

5.3.1. Resource Utilisation Profiles

To view the demand on the manufacturing resources, the utilisation curves were plotted. The tool generates the current utilisation of operator resources highlighting the daily peak levels in demand and when they occur. The demand on the production operators is shown in Figures 5.3a for pooling intervals of 1, 15 and 30 days. In this example, the production operators do not include QC/QA staff. Since QC/QA tasks require specialised personnel to carry out the process control activities, such operators are considered as belonging to another separate unit in the plant. Figures 5.3a illustrates that at a pooling interval of 1 day, a maximum of 9 production operators are engaged in the production activities at any time. On the other hand, a maximum of 5 operators are utilised for a 15-day pooling interval while the 30-day pooling strategy requires a maximum of 4 operators. The average utilisation of staff in all cases was also probed. This value corresponded to 6.0, 2.0 and 1.6 in the case of 1-, 15- and 30-day intervals respectively.

Examples of the demand on the QC/QA staff for successive batches are illustrated in Figures 5.3b for pooling intervals of 1, 15 and 30 days. Figures 5.3b indicates that typically, a maximum of 4 QC/QA staff are employed at any time in the process at a pooling interval of 1 day. At a longer pooling interval, Figures 5.3b shows that a maximum of 2 staff are used during the majority of the production time. The average utilisation of staff corresponded to 2.5, 1.5 and 1.3 in the case of 1-, 15-day and 30-day intervals respectively. The utilisation of QC/QA staff at a daily pooling interval was more intensive due to the fact that such operation necessitates more

parallel processing while more idle time is associated with a longer pooling interval. These results demonstrate the impact that different manufacturing options have on the demand for resources. Such utilisation performance metrics could be used to prompt a company to allocate the appropriate number of staff to carry out tasks efficiently depending on the manufacturing option selected.

The utilisation of USP and DSP equipment for two successive batches is plotted in the Gantt charts (Figures 5.4a-c) for pooling intervals of 1, 15 and 30 days respectively. The horizontal bars represent the duration of the equipment in use against time. In this example, the equipment can be in use for either the product-manufacture or equipment-preparation steps such as CIP and SIP. Since the two manufacturing strategies only differ in the downstream pooling sections, they possess similar USP equipment utilisation characteristics. As illustrated in Figure 5.4a, there are more lots being processed in the downstream section due to the need for daily pooling of eluate from the affinity chromatography in the 1-day option. Hence the DSP equipment is better utilised. In this case study, the next batch was initiated after the production fermentation had been completed. Hence there was more idle time between the completion of a current batch and the initialisation of the next batch. As explained previously, this situation was simplified to demonstrate the use of the tool to investigate different manufacturing options. Such charts can help to monitor the scheduling of resources and track the progress of the project.

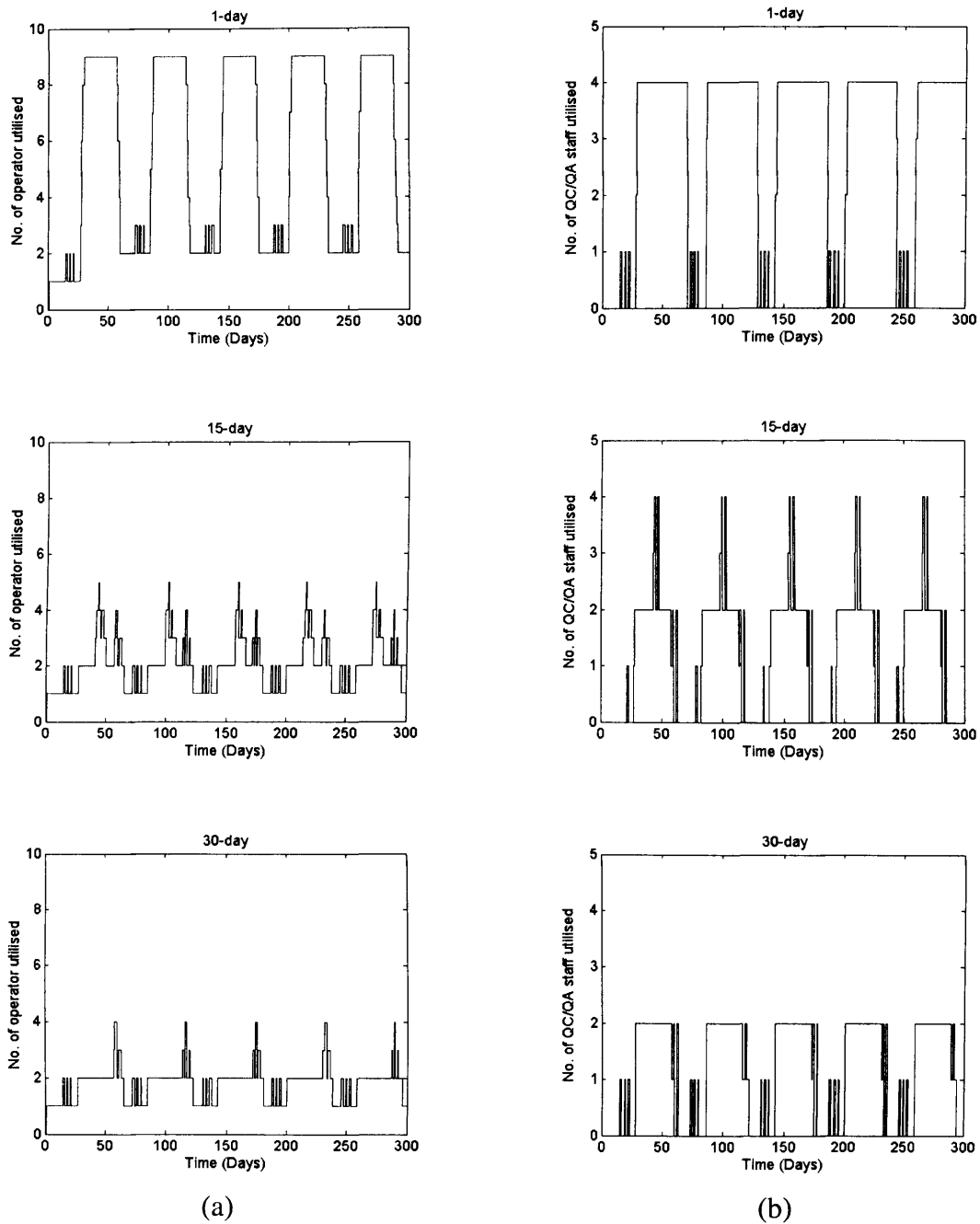
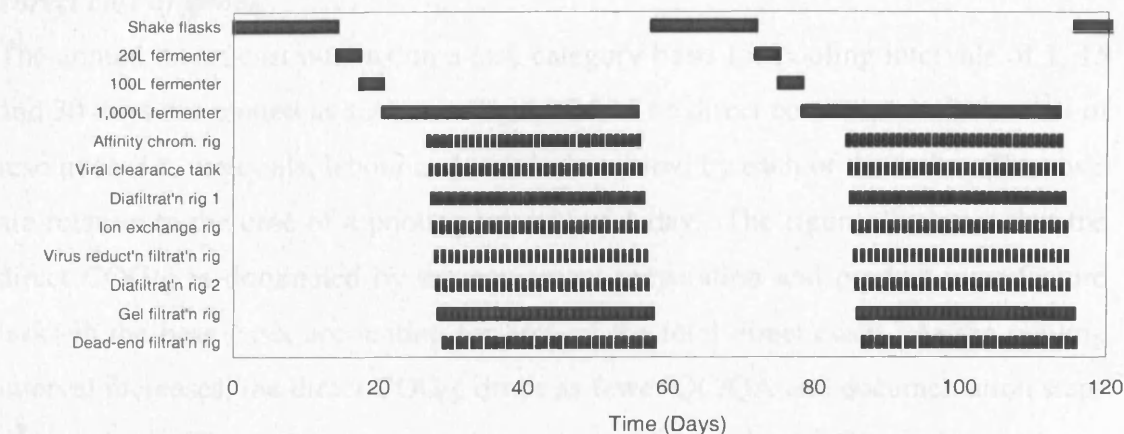
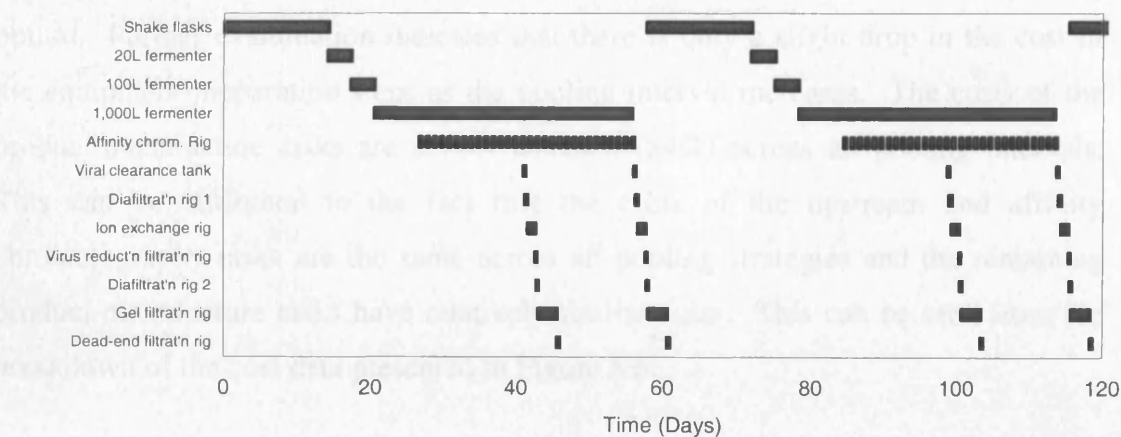


Figure 5.3. Utilisation of (a) production operators and (b) QC/QA staff for 1-, 15- and 30-day pooling strategy. The utilisation refers to the current utilisation of operator resources indicating the daily peak levels in demand.

(a)



(b)



(c)

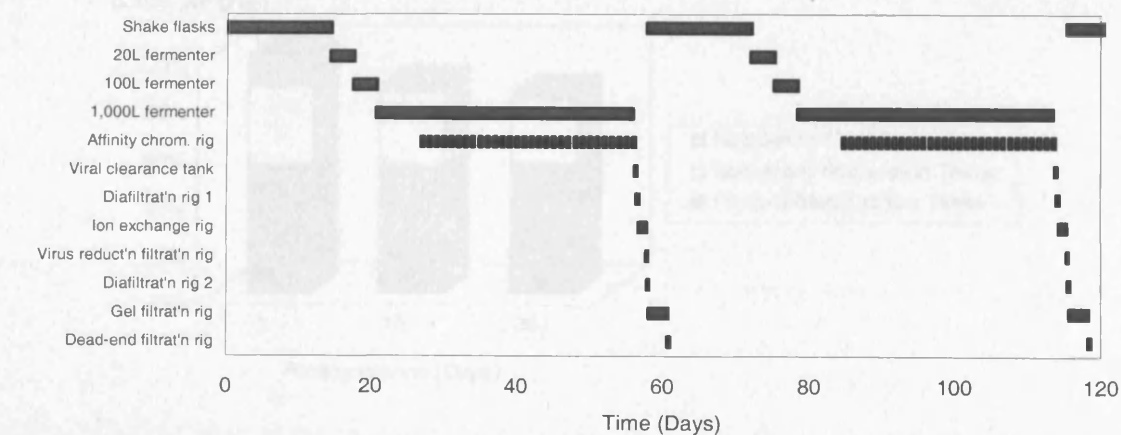


Figure 5.4. Gantt charts: Utilisation of equipment for (a) a daily (b) a 15-day and (c) a 30-day pooling strategy. The long bar indicates the equipment is used for a longer time while the short bar represents shorter usage time.

5.3.2. Economic Evaluation

Direct cost of goods

The annual direct cost outputs on a task category basis for pooling intervals of 1, 15 and 30 days are plotted as shown in Figure 5.5. The direct costs represent the cost of resources (i.e. materials, labour and utilities) required by each of the tasks. The costs are relative to the case of a pooling interval of 1 day. The figure illustrates that the direct COG/g is dominated by the equipment-preparation and product-manufacture tasks in the base case, accounting for 86% of the total direct costs. As the pooling interval increases, the direct COG/g drops as fewer QC/QA and documentation steps are required. These activities contribute about 14% of the COG/g in the base case and this falls sharply so that at an interval of 15 days, it is about 4%. This is due to the additional QC/QA and documentation tasks carried out during the 1-day pooling option. Further examination indicates that there is only a slight drop in the cost of the equipment-preparation steps as the pooling interval increases. The costs of the product-manufacture tasks are almost constant (54%) across all pooling intervals. This can be attributed to the fact that the costs of the upstream and affinity chromatography tasks are the same across all pooling strategies and the remaining product-manufacture tasks have relatively similar costs. This can be seen from the breakdown of the cost data presented in Figure 5.6.

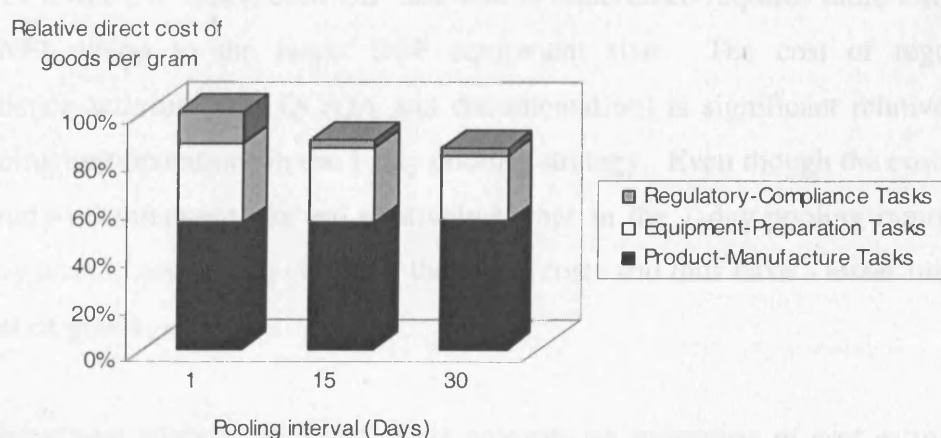


Figure 5.5. Annual direct COG/g on a task category basis for pooling intervals of 1, 15 and 30 days. Values are relative to a pooling interval of 1 day.

The annual direct COG/g can be broken down further to view the operational cost distributions of individual unit operations in the process flow. Figure 5.6 shows the direct costs (i.e. operators, materials, utilities) for each of the manufacturing tasks during the first year of production as a function of the pooling interval. The figure indicates that the production fermentation and CIP steps are the most expensive in all three pooling options. The high fermentation production costs are due to large usage of media and the associated labour over the extended culture cycle. This implies that the process development team must look for ways to cut down costs such as engaging in in-house media preparation rather than using ready-made media in disposable bags. Although the in-house media preparation is not considered in this analysis, options include making up and holding of such solutions in-house in either disposable or stainless steel equipment.

The costs of the upstream and affinity chromatography tasks are invariant with pooling interval due to the fact that the strategies differ only in the scale of the downstream recovery steps. Further examination illustrates that the affinity chromatography task contributes a substantial amount to the total direct COG/g. The costs of the buffer exchange/concentration task increase at longer pooling intervals as a result of the larger membrane filters used in such options. The figure indicates that the drop in CIP costs as the pooling interval increases is not as significant as might be expected. This can be attributed to the fact that although a longer pooling interval requires fewer CIP tasks, each CIP task that is undertaken requires more CIP buffer and WFI owing to the larger DSP equipment size. The cost of regulatory-compliance activities (i.e. QC/QA and documentation) is significant relative to the remaining unit operations in the 1-day pooling strategy. Even though the costs of the regulatory-compliance tasks are relatively higher in the 1-day pooling option, they are only a small proportion (14%) of the direct costs and thus have a lesser impact on the cost of goods.

The above cost analysis on a task basis provides an indication of cost estimates of specific tasks, thereby providing the capability to view where the running costs are concentrated and may be useful in focusing cost reduction efforts within a company.

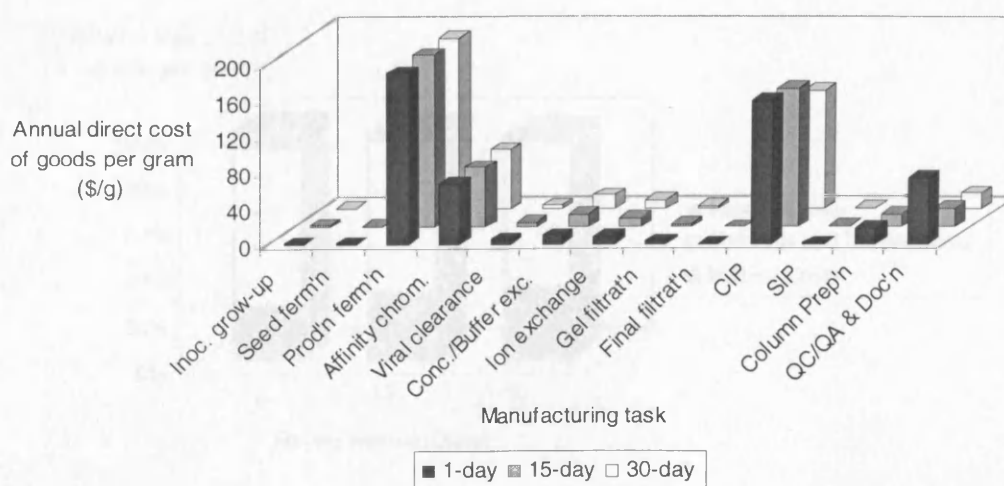


Figure 5.6. Breakdown of annual direct COG/g into each manufacturing task for pooling intervals of 1, 15 and 30 days.

Total cost of goods

The economics of the three pooling strategies were evaluated by the total COG/g, broken down into the indirect and direct costs (Figure 5.7). The costs are relative to the base case of a 1-day pooling option and show a close similarity for the three cases with the simulation result predicting the 30-day pooling option to have a slight cost advantage over the other two options with a 3% decrease in COG/g relative to the base case. Although longer pooling intervals have lower direct costs, the larger equipment scale contributes to high indirect costs, thus increasing the overall costs. A comparison of the indirect costs revealed a 7% increase for the 30-day pooling option. A 1-day pooling option has higher labour costs (8%) due to the frequent processing of the downstream steps. In order to compare the effect of different manufacturing strategies, such labour costs are computed based on their utilisation rather than considering them as a fixed annual cost. The main purpose of computing the labour costs based on utilisation was to highlight which manufacturing options were more labour-intensive. However, it should be emphasised that in industry, it is common practice to consider labour costs as fixed costs. The material and utilities category is the most significant contributor to the cost in each strategy.

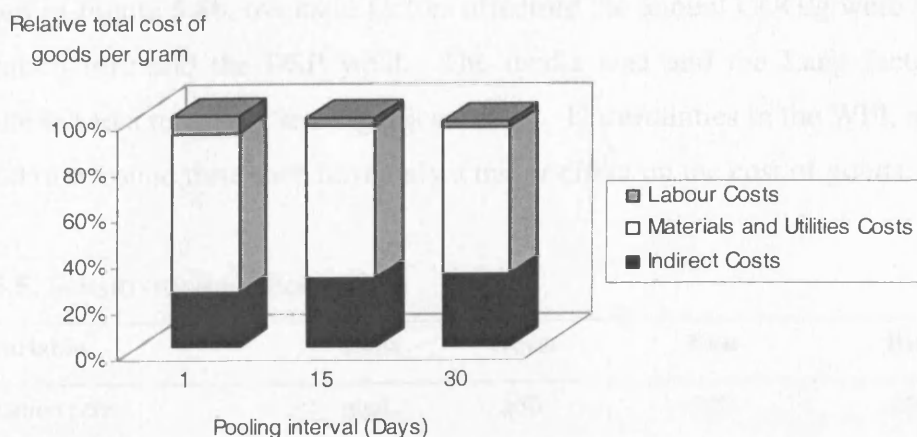


Figure 5.7. Total COG/g on a cost category for pooling intervals of 1, 15 and 30 days. Values are relative to a pooling interval of 1 day.

5.4. Sensitivity Analysis

As mentioned in Chapter 1, sensitivity analysis provides an organised and systematic way to examine the effects of parameters in a model on the key outputs. Accordingly the input variables in the case study were identified and subjected to $\pm x\%$ change (Table 5.5). The range of values for the input variables was determined from literature sources and through discussions with industrial experts (e.g. R. Francis, Protherics Plc., London, UK). In this case study, the company was assumed to produce a single Mab in the facility, and hence these values were likely variations between similar batches for a single product. A sensitivity analysis was then carried out for each of the variables in the extreme (worst and best) scenarios, keeping all other variables fixed at the baseline value.

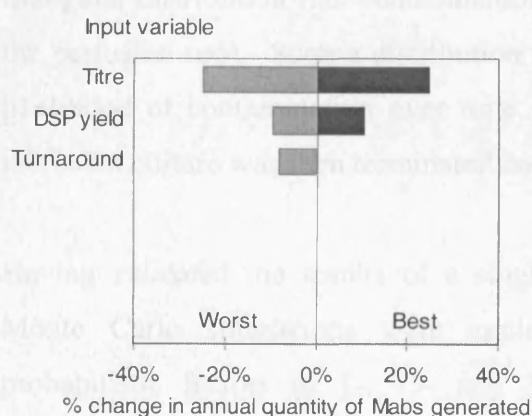
The range of outcomes for the annual product output and COG/g at a pooling interval of 15 days was monitored and displayed via Tornado plots (Figures 5.8a and b). Figure 5.8a indicates that the annual output is most sensitive to the fermentation titre, followed by the DSP yield. A 25% increase in fermentation titre results in a comparable increase in the amount of antibodies produced to 25 kg/year. On the other hand, a 25% reduction in the titre causes the annual output to drop to 15 kg/year. (Variables such as the Lang factor, media cost, water for injection (WFI) cost and operator salary, as expected, have no impact on the annual output.) As

illustrated in Figure 5.8b, the main factors affecting the annual COG/g were also the fermentation titre and the DSP yield. The media cost and the Lang factor each contribute but to a relatively less significant level. Uncertainties in the WFI, operator wage and turnaround time each have only a minor effect on the cost of goods.

Table 5.5. Sensitivity scenarios set-up.

Input variable	Units	Worst	Base	Best
Fermentation titre	mg/L	150	200	250
Downstream process yield	%	50	55	60
Turnaround time	days	5	2	1
Lang factor	-	7	6	5
Media cost	\$/L	25	20	15
WFI cost	\$/L	2	1	0.5
Operator wage	\$/h	35	25	15

(a)



(b)

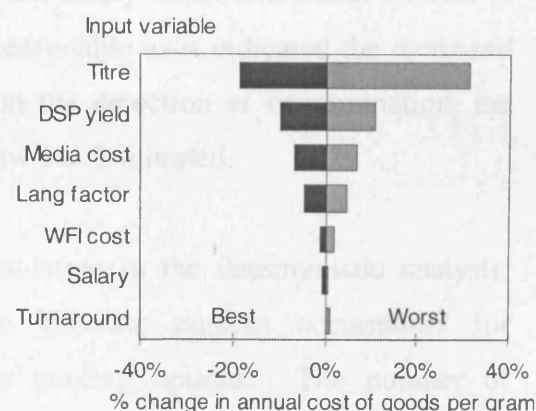


Figure 5.8. Tornado diagrams depicting the sensitivity of each variable of interest on (a) annual quantity of Mabs generated and (b) annual COG/g for a pooling interval of 15 days. The vertical axes intersect the horizontal axes at the baseline value.

5.5. Monte Carlo Analysis

5.5.1. Monte Carlo Set-up

In this study, fixed market demand and dose levels were assumed for the production of material for commercial use and were not considered as factors in the risk analysis. The fermentation titre was subjected to a variation of ± 50 mg/L and the DSP yield ranged between $\pm 5\%$ of the base value respectively (Table 5.6). These two variables were assumed to vary according to a discrete Normal distribution. Due to the long cycle times in perfusion culture, the possibility of contamination was a significant factor influencing the cost of goods and output. This risk was reflected in the model by adding a contamination probability associated with this mode of operation. The likelihood of contamination of 5% was incorporated into the model. The probability value indicated that the chance of a single perfusion run being contaminated was 5%. The *Input Random Number* block provided in Extend was used to generate random numbers according to the distributions specified in Table 5.6. When executed, the model randomly selected the particular day between Day 1 and Day 30 on which a contamination occurs according to a negatively-skewed triangular distribution (i.e. contamination is more likely to occur towards the end of the perfusion run). Such a distribution was reasonable as it indicated the increased likelihood of contamination over time. Upon the detection of contamination, the perfusion culture was then terminated and a new batch initiated.

Having validated the results of a single simulation in the deterministic analysis, Monte Carlo simulations were applied to generate random occurrences for probabilistic factors in 1-, 15- and 30-day pooling options. The number of simulation runs required to reach convergence was determined by monitoring the running averages of the simulation results until they levelled off. The number of simulation runs to reach convergence was 300. The outcomes from the Monte Carlo simulations were obtained so as to generate frequency distributions for the performance measures. The results for the different pooling interval strategies were then compared.

Table 5.6. Monte Carlo simulation set-up: Input risk parameters and their discrete probability distributions.

Variable	Units	Value	Probability
Fermentation titre	mg/L	150	0.1
		175	0.2
		200	0.4
		225	0.2
		250	0.1
DSP yield	%	50	0.1
		53	0.2
		55	0.4
		58	0.2
		60	0.1
Perfusion run contaminated?	-	Yes	0.05
		No	0.95

5.5.2. Monte Carlo Simulation Results and Discussion

Figures 5.9a and b illustrate the frequency distribution plots and cumulative frequency curves for the annual quantity of Mabs generated and COG/g at a pooling interval of 15 days respectively. From the deterministic results, given a titre of 200mg/L, the company may expect that the typical quantity of Mabs generated in their facility in a year to be 20kg after purification. It was assumed that the process output was sufficient to satisfy initial market demand. However, by considering uncertainties in titre and DSP yield together with the risk of contamination, the data in Figure 5.9a indicates that although the modal annual output is 20.0kg, the computed mean is only 19.3kg. This demonstrates that the impact of contamination is skewing the mean to the left of the modal value, consequently producing a lower value for the expected amount of Mabs generated.

In the deterministic case, the annual output of 20kg was achieved without considering the occurrence of risk or failure. With a contamination probability of 5% in the process, a more rational target of at least 19kg is chosen for the purpose of

discussion. It can be seen that the probability of achieving an annual output of at least 19kg is 0.88. Hence, there is a 12% chance that the output would fall below 19kg. These results highlight the limitations of relying on deterministic outputs for decision-making in processes subject to randomness and risk. The company may seek to improve the process in order to raise the output given the inherent randomness expected and to gain confidence in the ability to deliver the target amount of Mabs product.

Figure 5.9b shows that there exists a corresponding range of possible outcomes for the COG/g. Such results can be used to determine the likelihood of exceeding a particular COG/g limit. As illustrated in Figure 5.9b, the likelihood of exceeding the cost obtained by deterministic methods is about 68% and again reinforces the dangers of using single-point forecasts. A company should take into account such variations in costs into their financial planning.

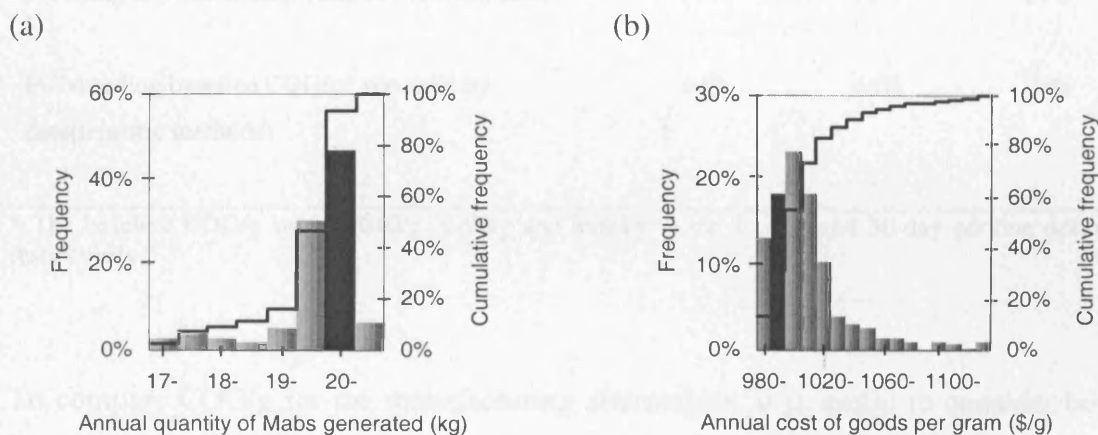


Figure 5.9. Frequency distribution plots and cumulative frequency curves for (a) annual quantity of Mabs generated and (b) annual COG/g for a pooling interval of 15 days. The left axes correspond to the number of occurrences (histogram) and the right axes refer to the cumulative percentage (line). The solid column indicates the deterministic base value.

Table 5.7 summarises the results of the Monte Carlo simulation for the three different pooling strategies. The results are ranked in order with the best first. The 1-day pooling strategy offers the lowest likelihood of failing to meet the market demand and of exceeding the baseline cost budget as compared to the other two alternatives when operated under uncertainty. Only the 30-day pooling process has an expected production mean below the 19kg target. This pooling strategy is the riskiest in terms of the likelihood of failing to meet demand and of exceeding the baseline COG/g.

Table 5.7. Summary of key results for the Monte Carlo analysis of the 3 alternative strategies.

Statistics	Pooling option		
	1-day	15-day	30-day
Expected annual output (kg)	19.4	19.3	18.9
P(Failing to yield at least 19kg of Mabs annually)	11%	12%	24%
P(Exceeding baseline COG/g* obtained by deterministic methods)	64%	68%	70%

* The baseline COG/g was \$1,010/g, \$990/g and \$980/g in the 1-, 15- and 30-day pooling options respectively.

To compare COG/g for the manufacturing alternatives, it is useful to consider both the expected costs and associated risks. Figure 5.10 depicts the risk (standard deviation) against the expected annual cost of goods per gram for each pooling strategy. The choice between long and short pooling intervals would require a trade-off between the cost budget and risk of each strategy be established. Options with low expected costs and risks are preferred. Although the deterministic analysis predicted that the 30-day pooling strategy has a slight cost advantage over the other two alternatives, the Monte Carlo simulation results indicate that the three pooling options have comparable expected annual COG/g. It is apparent that the 30-day pooling strategy is the least favourable of the options because of its higher risk

(larger standard deviation). The COG/g outcomes for the 30-day option are more disperse and hence this option carries a greater risk.

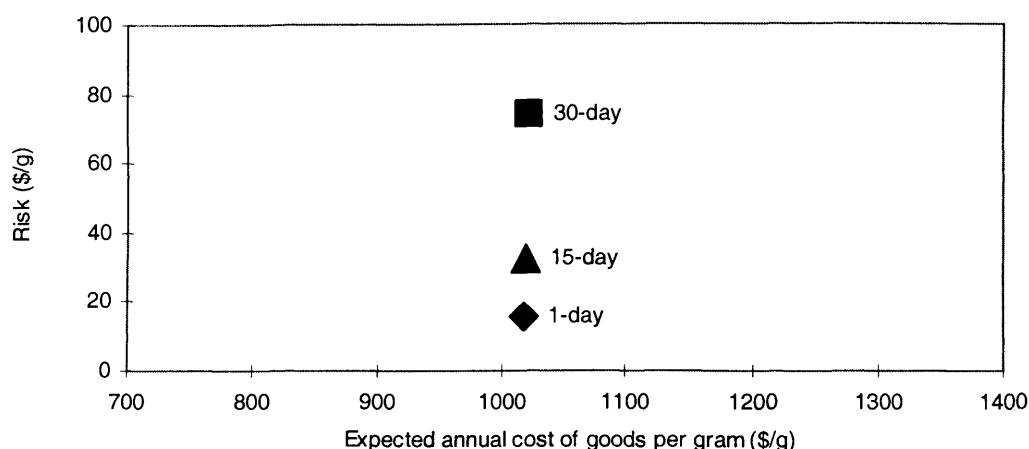


Figure 5.10. Plot of the risk versus the expected annual COG/g for each pooling strategy. The risk refers to the standard deviation of the cost of goods distributions. The three pooling options have comparable expected annual COG/g of \$1,020/g.

Based on the COG performance metric, the company would probably opt for the 1- or 15-day pooling option with their associated lower risks. The final decision may relate to the utilisation of DSP resources. Since DSP activities are performed less frequently with a longer pooling interval, this potentially leads to more idle capacity in the DSP purification train. On this basis, the 1-day pooling strategy with a smaller manufacturing plant seems more favourable since the DSP equipment is better utilised.

5.6. Scenario Analysis

Scenario analysis is a process of analysing possible future events by considering alternative possible outcomes (scenarios). The basic principle of scenario planning is to understand as best as possible likely trends and to make strategic decisions based on an analysis of the consequences of the most likely scenarios. The analysis is designed to allow improved decision-making by allowing more complete consideration of outcomes and their implications.

5.6.1. Scenarios Set-Up

The following possible scenarios were envisaged and verified through discussions with industrial experts. The tool was used to run these scenarios independently. The same stochastic analysis was performed to evaluate the impact on key performance metrics and determine the ranking of the three pooling alternatives under different scenarios.

a) The product is unstable and the product degrades as the holding time increases.

In the previous base-case scenario, the product was assumed to be stable and all three pooling strategies achieved similar product titres. The first alternative scenario examined the possibility that the product was unstable and hence it would not be possible to achieve the same titre levels in all three options. This is reasonable as the product degrades as the holding time increases. In this case, the following modifications were made to the models. A 20% reduction in the average recoverable product titre (i.e. 160mg/L instead of 200mg/L) for the 30-day pooling option was assumed (A. Sinclair, Biopharm Services Ltd, Chesham, UK; R. Francis, Protherics Plc., London, UK). As for the 15-day pooling option, the average recoverable titre was reduced by 10% to 180mg/L. For the Monte Carlo analysis, a discrete normal distribution was applied to both cases. The probability distribution for the fermentation titre of the 1-day pooling option remained unchanged. The changes in titre values for the scenario are summarised in Table 5.8. Again, the *Input Random Number* block was employed to generate random numbers according to the distributions.

Table 5.8. Changes to inputs for scenario ‘a’.

Pooling option	Possible titre values (mg/L)	Probability
15-day	130	0.1
	155	0.2
	180	0.4
	205	0.2
	225	0.1
30-day	110	0.1
	135	0.2
	160	0.4
	185	0.2
	210	0.1

b) The company has existing downstream equipment in their facility and the product is stable.

The second scenario considered the situation where the biopharmaceutical company had an existing downstream purification suite in their plant. The company wished to assess the most appropriate pooling strategy (i.e. 1-, 15- or 30-day intervals), given a fixed downstream process scale. In this case, the downstream equipment scale of the 1-day pooling option was assumed and the pooling interval was then varied to 15 and 30 days. With an existing facility, multiple cycles or longer cycle times are likely to be used as the harvest volume increases rather than linear scaling of column volume or membrane area (Personal communication: R. Spurling, Biopharm Services Ltd, Chesham, UK). This scenario examined the impact of such changes. The downstream process yields remained unchanged and the product was assumed to be stable.

c) The product is unstable and the company has existing downstream equipment in their facility.

The last scenario was a combination of scenarios ‘a’ and ‘b’. The company was assumed to have a fixed downstream equipment scale in their facility. In addition, the product was considered to be unstable.

5.6.2. Scenarios Results and Discussion

Table 5.9 summarises key statistics used to compare the process alternatives in each scenario. The ranking of the results in each scenario is also tabulated in the table. The plots of risks versus the expected COG/g for each pooling alternatives in the different scenarios are shown in Figures 5.11a-c.

Table 5.9. Summary of simulation results for the different scenarios.

Scenario	Statistics	Values			Ranking order (best first)
		1-day	15-day	30-day	
Unstable product	Expected annual output (kg)	19.4	17.4	15.4	1, 15, 30
	Expected COG (\$/g)	1020	1120	1230	1, 15, 30
	P(Failing to yield at least 19kg of Mabs annually)	11%	100%	100%	1*
Stable product; same DSP equipment scale	Expected annual output (kg)	19.4	19.0	18.1	1, 15, 30
	Expected COG (\$/g)	1020	840	810	30, 15, 1
	P(Failing to yield at least 19kg of Mabs annually)	11%	15%	28%	1, 15, 30
	P(Exceeding baseline COG/g obtained by deterministic methods for each strategy)	64%	71%	75%	1, 15, 30
Unstable product; same DSP equipment scale	Expected annual output (kg)	19.4	17.0	14.8	1, 15, 30
	Expected COG (\$/g)	1020	900	1080	15, 1, 30
	P(Failing to yield at least 19kg of Mabs annually)	11%	100%	100%	1*

* In these cases, the 15- and 30-day pooling options fail to meet the output criterion and hence are not feasible.

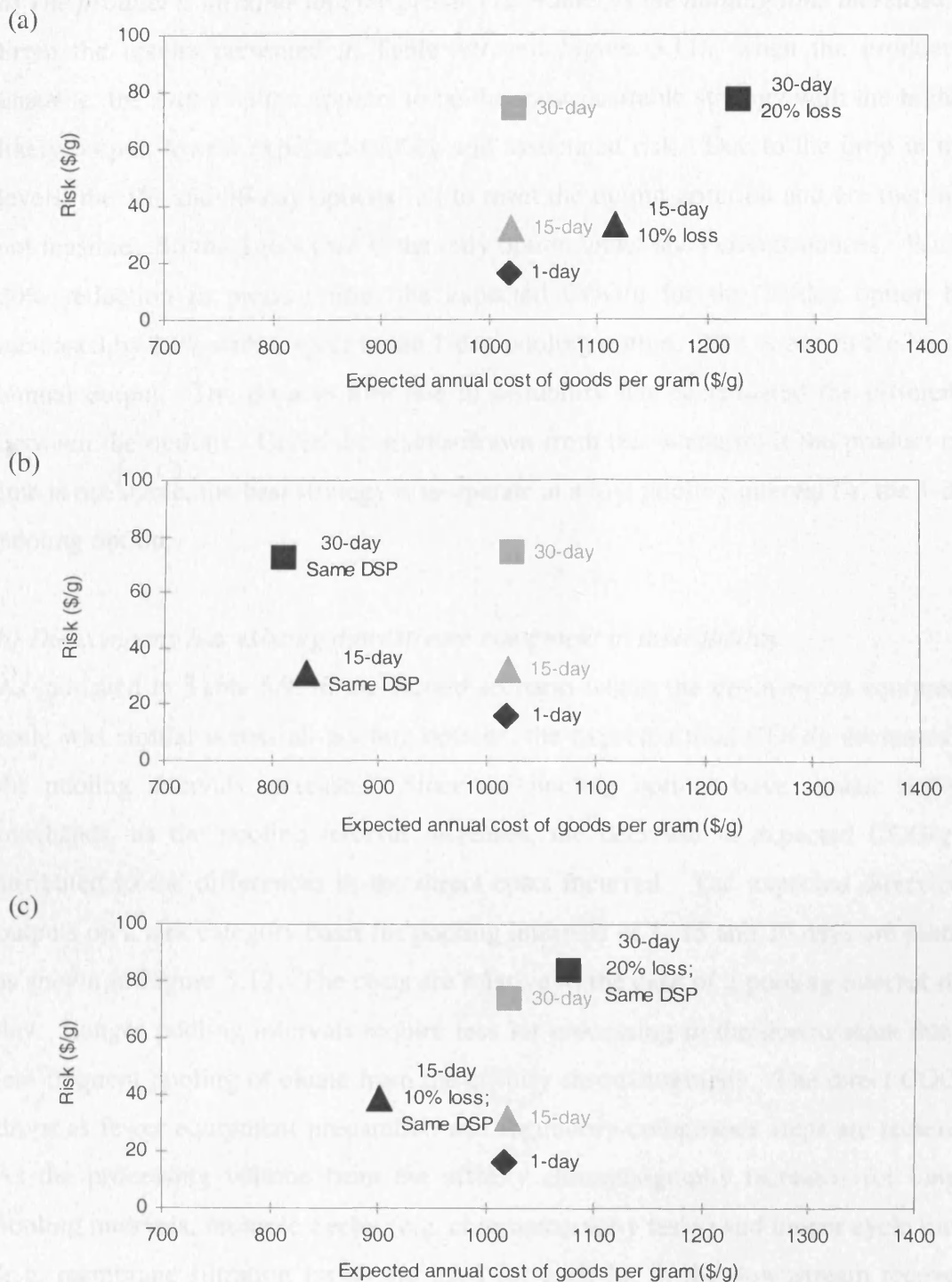


Figure 5.11. Plot of the risk versus the expected annual cost of goods per gram for the different scenarios examining the effects of (a) an unstable product, (b) fixed DSP equipment across all pooling options and (c) combination of scenarios 'a' and 'b'. The grey symbols represent the simulation results from the original Monte Carlo base case analysis. The risk refers to the standard deviation of the cost of goods distributions.

a) The product is unstable and the product degrades as the holding time increases.

From the results presented in Table 5.9 and Figure 5.11a, when the product is unstable, the 1-day option appears to be the most desirable strategy with the highest likely output, lowest expected COG/g and associated risk. Due to the drop in titre levels, the 15- and 30-day options fail to meet the output criterion and are therefore not feasible. So the 1-day case is the only option under such circumstances. With a 20% reduction in product titre, the expected COG/g for the 30-day option has increased by 23% with respect to the 1-day pooling option. This is due to the loss in annual output. The drop in titre due to instability has accentuated the difference between the options. Given the results drawn from this scenario, if the product cell line is not stable, the best strategy is to operate at a low pooling interval i.e. the 1-day pooling option.

b) The company has existing downstream equipment in their facility.

As indicated in Table 5.9, in the second scenario where the downstream equipment scale was similar across all pooling options, the expected total COG/g decreases as the pooling intervals increases. Since the pooling options have similar indirect overheads, as the pooling interval increases, the decrease in expected COG/g is attributed to the differences in the direct costs incurred. The expected direct cost outputs on a task category basis for pooling intervals of 1, 15 and 30 days are plotted as shown in Figure 5.12. The costs are relative to the case of a pooling interval of 1 day. Longer pooling intervals require less lot processing in the downstream due to less frequent pooling of eluate from the affinity chromatography. The direct COG/g drops as fewer equipment-preparation and regulatory-compliance steps are required. As the processing volume from the affinity chromatography increases for longer pooling intervals, multiple cycles (e.g. chromatography tasks) and longer cycle times (e.g. membrane filtration tasks) are used for each lot in the downstream recovery steps, thereby reducing the number of equipment-preparation steps. In addition, with less lot processing, operating at longer pooling intervals results in reduced regulatory-compliance overheads since such tasks are executed at the end of each lot rather than each cycle. It is apparent that the 1-day pooling strategy is inferior to the other two options, because of the higher annual running costs. The significant cost difference can be attributed to the increased lot processing in the downstream section at lower pooling intervals. The 15- and 30-day strategies are seen to offer significant

reductions (18% and 21% respectively) in operating costs relative to the 1-day pooling case. However, the 1-day pooling option possesses improved utilisation of DSP equipment since such strategy processes more lots in the downstream section due to the daily processing of eluate from the affinity chromatography.

In the plot of risk against the expected cost, the results are conflicting. Examining the data in Figure 5.11b indicates that the 30-day option has the lowest expected COG/g. However, this option possesses the highest risk. On the other hand, the 1-day pooling option has the lowest risk but highest expected cost. The expected COG/g results between the 15- and 30-day options are close, though the 30-day option offers a slight advantage. However, as seen in Table 5.9, the 30-day option fails to meet the output criterion of 19kg and is therefore rendered non-feasible. The 15-day strategy emerges as the more favourable option, which meets the output target. In this particular scenario, the simulation results suggest that given the fixed DSP capacity of the 1-day pooling option, the strategy of pooling every 15 days combined with maintaining the output target is more practical.

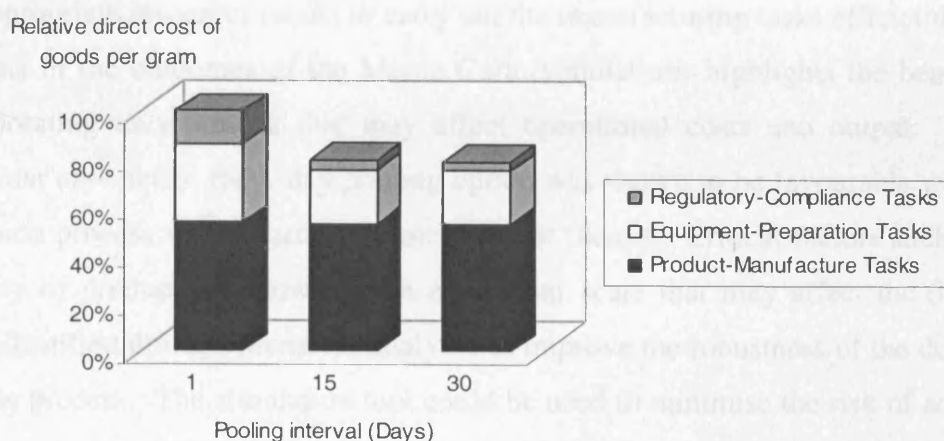


Figure 5.12. Expected annual direct COG/g on a task category basis for pooling intervals of 1, 15 and 30 days. The pooling options have the same DSP equipment scale. Values are relative to a pooling interval of 1 day.

c) The product is unstable and the company has existing downstream equipment in their facility.

Examining the cost data in Table 5.9 reveals that the 15-day option is ranked first based on the COG/g criterion. However, the 15- and 30-day pooling options fail to meet the output criterion and are therefore not viable. Given the existing operating conditions, the 1-day option appears to be the only solution. These results again indicate that the 1-day pooling strategy is the most appropriate option when the product cell line is unstable.

5.7. Conclusions

The case study demonstrates the functionalities of the tool to output the cost of goods on a task basis, view the demands on resources and compare the effect of QC/QA and batch documentation activities under different production strategies. The simulation results show the cost reduction of QC/QA overheads at longer pooling intervals. The results drawn from the study provide indications of the operational costs incurred to accomplish project execution. The tool also generates indication of the appropriate resources (staff) to carry out the manufacturing tasks efficiently. The analysis of the outcomes of the Monte Carlo simulations highlights the benefits of incorporating uncertainties that may affect operational costs and output. In this particular case study, the 1-day pooling option was shown to be favourable when the perfusion process was subject to numerous risk factors. Critical factors such as the stability of product and downstream equipment scale that may affect the decision were identified through scenarios analyses to improve the robustness of the decision-making process. The simulation tool could be used to minimise the risk of adopting an inappropriate operating strategy and enhance the quality of decision-making within a company. The application of such a tool is taken a step further in the next chapter to compare the economic feasibility of fed-batch and perfusion cultures.

The configuration of the tool enabled the scenarios set out in this case study to be evaluated efficiently. Customised features for user-defined probability distributions of uncertain parameters were incorporated so as to provide the capability to represent the inherent randomness in biomanufacturing. Such a tool facilitated the Monte

Carlo simulation approach to be carried out by specifying the relevant input probability distributions for each of the process uncertainties. The tool output the simulation results from each trial run to Excel and hence generated a range of possible outcomes. This enabled the plotting of the frequency distributions of key output parameters and the tabulation of various useful statistical summaries. The main disadvantage of the tool was the lengthy computational time required to complete a Monte Carlo simulation run. The latter issue is addressed in the further work section in Chapter 7.

CHAPTER 6

A Further Application: Fed-batch versus Perfusion Culture

6.1. Introduction

Recombinant expression in mammalian cell cultures is the principal means for the commercial production of therapeutic proteins such as monoclonal antibodies (Mabs). The cultivation of such cultures can be operated in fed-batch or perfusion modes. The fed-batch operation has been widely adopted due to its operational simplicity. However, considerable progress has been made to improve the operation of perfusion cultures, thus making large-scale perfusion an increasingly feasible option for cell culture. To date, limited research however has been published to compare the production economics of fed-batch and perfusion culture. In this chapter, the simulation tool, *BioPharmKit*, was employed to evaluate the economic feasibility of both culture modes via a case study. The case study is based upon the large-scale production of a therapeutic Mab. The study aims to investigate the relative economics of the two operational modes through the application of the tool by examining key performance metrics such as the cost of goods per gram (COG/g) and the net present value (NPV). Other parameters of interest include the capital investment, media consumption and the resource utilisation profiles.

The decision-making situation is often characterised by uncertainties arising from the manufacturing process. Incorporating the effects of risk analysis would help to estimate the potential risks associated with probabilistic factors that may impact key performance measures. Decision trees and Monte Carlo simulations are typical tools used for risk analysis (Doctor *et al.*, 2001). The decision tree technique provides a highly effective framework where possible options are laid out with their values of outcomes and associated probabilities of achieving them. Such a technique provides a simple but useful method to compute the expected values of output parameters and make decisions based on such values. The expected value of each option is computed by multiplying the outcome with the probability in each path and then adding the weighted values of all the paths. However, the decision tree approach does not explicitly acknowledge the range of uncertainty and is not ideal for events that could happen simultaneously. For more complicated problems, the Monte Carlo simulation technique is a practical way of specifying the input probability functions of uncertain factors to imitate the randomness inherent in biopharmaceutical manufacture. Sample values are randomly generated by using the input probability distribution for each uncertain quantity and then used to determine a trial outcome for the model. Such a sampling process leads to a range of possible outcomes for a desired measure of metric. These can be used to generate the frequency probability distributions of designated outputs and key statistics such the likelihood of not achieving or exceeding a critical threshold value. The relative ease of using such risk analysis methods for decision-making is also investigated in this chapter.

For this chapter, Section 6.2 focuses on the case study that describes the fed-batch and perfusion modes of operation used for the commercial production of Mabs. A detailed analysis of the deterministic simulation results is provided in Section 6.3. In Section 6.4, the uncertainties in the process are identified and a sensitivity analysis carried out to investigate the impact of influencing factors on key output parameters. In Section 6.5, a risk assessment is performed using a simple decision tree method to imitate the randomness inherent in the process. A Monte Carlo simulation technique is also employed to provide a more complete estimation of the overall uncertainty so that the possible range of outcomes can be obtained and the resulting frequency distributions can be used to make probabilistic statements about the original problem. Lastly, a conclusion is provided in Section 6.6.

6.2. Case Study Background

6.2.1. Case Study Set-up

The examples developed in this chapter are based on a biopharmaceutical company developing a therapeutic Mab expressed in mammalian cells for commercial use. The company faces the question of whether to adopt fed-batch culture in the manufacturing facility or to opt for a plant utilising perfusion culture. During early phase process development, a company may expect to have little supporting data regarding the feasibility of fed-batch and perfusion cultures but they may wish to investigate the differences between such operational modes. The case study was used to explore the functionalities of the tool to provide cost estimates, perform mass balance calculations, simulate resource handling, and evaluate potential risks so as to predict the feasibility of fed-batch and perfusion cultures. The potential advantages and disadvantages of the two operational modes are highlighted in Chapter 2. The decision to manufacture the therapeutic Mab using the fed-batch operation typically requires an investment that is largely front-loaded owing to the need for a larger bioreactor capacity. The use of perfusion culture is attractive since such an operational mode can achieve a higher productivity in a relatively small size bioreactor as compared to fed-batch culture. However, perfusion processes can suffer from increased contamination and equipment failures due to the extended culture cycle.

The relative merits and limitations of these two operational modes are explored with the help of technical and financial indicators. The key performance metrics used to compare the manufacturing strategies were the utilisation profiles of resources and COG/g. The cost analysis was extended to include other profitability indicators such as the NPV and the internal rate of return (IRR). Other key outputs included the capital investment, payback period and the levels of media consumption.

Table 6.1 summarises the key assumptions in process inputs between fed-batch and perfusion modes of operation validated through discussions with industrial experts (e.g. B. Fish, Cambridge Antibody Technology, Cambridge, UK; J. Wayte, Lonza Biologics, Slough, UK and R. Francis, Protherics Plc., London, UK). As indicated in

the table, a more extensive seed fermentation train is needed with fed-batch culture due to the larger volume of the final production bioreactor. By comparison the continuous perfusion systems have shorter seed trains but increased operational cycle times and lower titres. Both processes were designed to produce 51kg of a Mab product per year, which is a typical industrial figure. The duration of the production fermentation for the fed-batch and continuous perfusion processes were given typical average values of 15 and 35 days respectively. Typical antibody titres in fed-batch cultures are in the range of grams per litres, with 1g/L being the norm (Personal communication: J. Wayte, Lonza Biologics, Slough, UK). The fed-batch process was assumed to have a titre of 1g/L. In order to achieve the same annual product output as the fed-batch option, the fermentation titre of the perfusion process was designed to be 0.35g/L. Such a titre value was reasonable, as discussed in the literature review provided in Chapter 2. In this case study, emphasis was placed on the application of the tool to compute cost estimates, perform mass balance calculations and carry out risk analysis. While sensible inputs were sought, the main purpose was to demonstrate the functionalities of the tool to assess manufacturing alternatives, hence the simulation results were not definitive answers.

Table 6.1. Key assumptions for fed-batch and perfusion modes of operation.

Assumption	Fed-batch	Perfusion
Size of production bioreactor (L) (working volume)	5,000	1,000
Seed bioreactor train (L)	20; 100; 500	20; 100
No. of production bioreactors	1	1
Duration of production fermentation (days)	15	35
Fermentation titre (g/L)	1.0	0.35

Figure 6.1a illustrates the process flowsheet for the production of a therapeutic Mab from mammalian cell culture produced by a fed-batch mode of operation. There are a number of feeding approaches for a fed-batch process operation, each of which affects the initial starting volume. Typically strategies involve one or two continuous feeds of 10-50% of the final reactor volume added over a period corresponding to

40-70% of the total process duration. In this case study, one continuous feed volume of 30% added over approximately 65% of the total process time was considered, i.e. 3,500L initial volume with addition of 1,500L over 10 days. Centrifugation was then used for the removal of cells from the product-containing media. This was followed by a series of purification steps.

Figure 6.1b depicts the production process for the antibody-based product using a perfusion mode of operation. A continuous perfusion rate of 1 reactor volume per day was assumed. During the perfusion culture, cells were retained in the bioreactor and liquor containing the product was collected. The collection of the product-enriched liquor commenced after 5 days when a constant growth rate was achieved. The harvested liquor collected daily was loaded onto the affinity column, eluted as purified product and stored in disposable bags. The concentrated eluate from 15 successive chromatography runs was then pooled and passed through the other purification steps comprising the complete process.

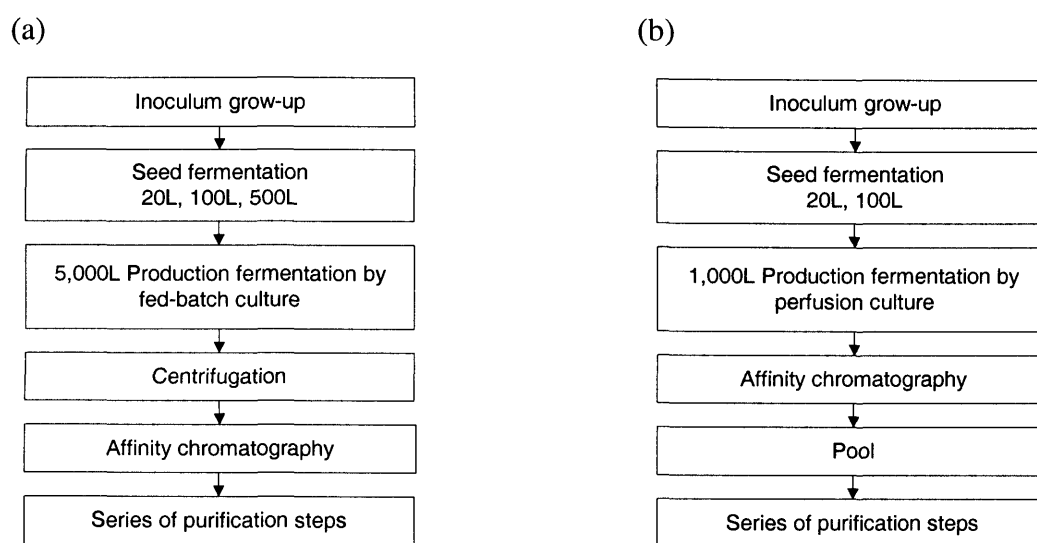


Figure 6.1. An overview of the mammalian-based process flowsheet for Mabs production using (a) fed-batch and (b) perfusion culture. In each case, the recovery stages are assumed to have the same product yield.

Besides these product-manufacture tasks, ancillary activities such as equipment-preparation (e.g. cleaning-in-place (CIP), sterilising-in-place (SIP)) and regulatory-compliance (e.g. quality control (QC), quality assurance (QA) and batch documentation) ran simultaneously during the simulation in both modes of operation. The CIP task for the equipment resource and the regulatory-compliance steps was invoked after the completion of each unit operation. The SIP task for certain equipment was activated prior to the start of the unit operation.

Both processes were designed to use the same sequence of purification steps and to have similar equipment sizes (Table 6.2). Since the fed-batch and perfusion systems have almost similar downstream steps, it was assumed that equivalent overall recovery yields were achieved. There was only one production train in each manufacturing option. The plant would operate seven days a week and 48 weeks a year. In the fed-batch case, a new batch was initialised after the completion of the inoculum grow-up stage (allowing for turnaround). For the perfusion case, the duration of the production fermentation step (35 days) was more than double that of the fed-batch case (15 days); hence the new batch was initialised such that the seed fermentation sequence would finish just in time for the production fermenter to be available again. This scenario reflected a typical situation in industry where scheduling is set so as to fully utilise the process equipment. A simplification for the case study was that media and buffer arrived pre-made and sterile in disposable bags. In this case study, the aspects of product stability were not dealt with.

Table 6.2. Downstream equipment sizes for the fed-batch and perfusion systems.

Manufacturing task	Resource pool name	Size*
		Fed-Batch/Perfusion
Affinity chromatography	Affinity chromatography rig & column	V = 45L; H = 15cm; D = calculated
Chemical retrovirus inactivation	Virus inactivation tank	V = 1,500L
Concentration/Buffer exchange 1	Diafiltration pellicon cassettes	A = 20m ²
Ion exchange chromatography	Ion exchange chromatography rig & column	V = 45L; H = 15cm; D = calculated
Virus reduction nanofiltration	Virus reduction filter	A = 3m ²
Concentration/Buffer exchange 2	Diafiltration pellicon cassettes	A = 30m ²
Gel filtration chromatography	Gel filtration chromatography rig & column	V = 80L; H = 50cm; D = calculated
Final filtration	Cartridge filter	A = 2m ²

* Verified through discussions with industrial experts (e.g. R. Francis, Protherics Plc., London, UK; J. Savery, J. & R. Spurling, Biopharm Services Ltd., Chesham, UK)

6.2.2. Methods

In this case study, the perfusion model built in the previous chapter based on a pooling interval of 15 days was employed. The input value for the fermentation titre was changed to 0.35g/L. For the fed-batch model, the same model was employed with some modifications. The perfusion block was replaced by the customised fed-batch fermentation block with a fermentation titre of 1.0g/L. A centrifugation unit operation was connected to the output connector of the fed-batch production fermentation block. An additional seed train block was cloned from the library and connected to the USP sequence. The equipment-preparation tasks of CIP and SIP were created for this seeding step. The sizes of the seed train were updated according to the specifications in Table 6.1.

The decision-support tool, *BioPharmKit*, developed in Extend was used to model the two scenarios independently and the results compared at the end of the simulations. Initially deterministic cases were simulated to establish the values of the base case output parameters. The simulation outputs were again verified with industrial experts to validate the results. The deterministic analysis does not account for risk factors that may affect the performance metrics (i.e. annual output, COG/g and NPV), which are pertinent to the process of decision-making. To take into account the inherent variability of process factors, the key uncertainties in the system were identified and a sensitivity analysis was then performed to investigate the impact on output parameters. The uncertainties were the fermentation titre, process yield, Lang factor, media cost, CIP reagents cost and processing reagents cost, which were used in the approach to determine the sensitivity of the base case to variability in process parameters. Each of these variables was subjected to $\pm x\%$ change, keeping all other variables at the baseline values.

Both the decision tree and Monte Carlo simulation techniques were then employed to incorporate risk factors such as contamination and filter fouling. The decision tree method was used to provide a simple and efficient evaluation of manufacturing alternatives by incorporating the probabilities of contamination and filter fouling. The probable scenarios were envisaged and the tool was used to run each of these scenarios independently. For example, one possible scenario involved the situation where only one bioreactor run was being contaminated. The risk-adjusted values in each probabilistic case were determined and set-up using a decision tree in an Excel spreadsheet so as to compute the expected values. To simplify the decision tree analysis, process uncertainties (e.g. fermentation titres and downstream process (DSP) yields) were given typical average values. In order to provide a more complete estimation of such uncertainties, a Monte Carlo analysis was then performed to incorporate the randomness in biopharmaceutical manufacture subject to fluctuating fermentation titres and process yields. Probability distributions were assigned to these inputs and simulations were run to determine the distribution of possible outcomes for the outputs. The simulation results from each method were analysed in order to compare the relative usefulness and limitations of these two risk analysis methods for decision-making.

6.3. Deterministic Results and Discussion

The tool predicted that the fed-batch plant would be able to achieve 19 bioreactor runs (19 lots) per year, as opposed to 9 bioreactor runs in the perfusion case. The lower number of runs in the perfusion option can be attributed to the longer fermentation cycle. By pooling the concentrated eluate from 15 successive chromatography runs in the perfusion case, the plant generated a product output of 18 lots per year.

6.3.1. Resource Utilisation Profiles

Figures 6.2a and b depict the media utilisation over time for fed-batch and perfusion operation respectively. Figure 6.2a indicates a variable demand for media during successive fermentation runs in the fed-batch system. The spiky peaks during the start of the fed-batch process are due to the sequence of build-up seed steps and the high initial starting volume assumed followed by a gradual addition of fresh medium. Figure 6.2b highlights a constant demand on media over the bioreactor runs in the perfusion process. The simulation results predicted a 2.6 fold increase in media consumption in perfusion relative to fed-batch operation. This is due to the need in perfusion for a continual addition of a steady volume of media over the extended fermentation cycle time. This is one factor that has implications for the direct costs of the two processes.

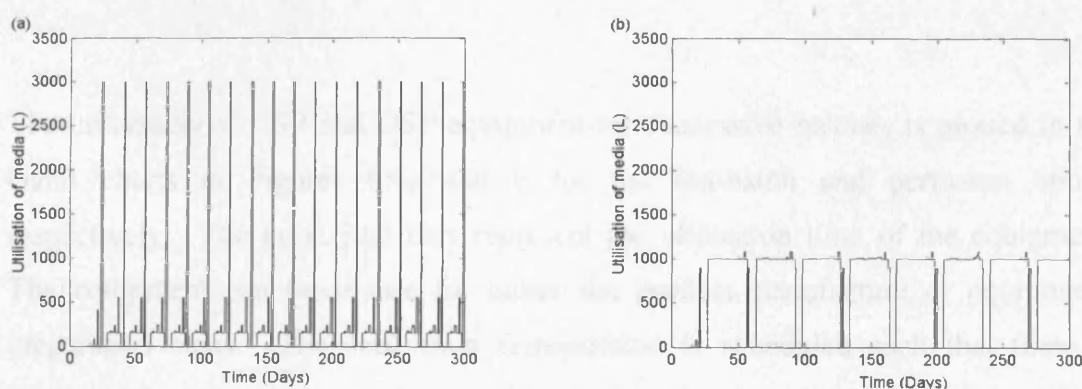


Figure 6.2. Utilisation of media for the (a) fed-batch and (b) perfusion mode of operation. The curves show more spiky peaks in demand for media in the fed-batch system.

Examples of the demand on the production operators for successive batches are illustrated in Figures 6.3a and b for the fed-batch and perfusion processes respectively. The average utilisation of staff is 3.4 and 3.6 operators respectively but demand on the production operators is highly intermittent. The graphs highlight the level of peak demand and when they occur. In practice these peak demands could be accommodated with four operators and occasional double-shift working, balanced by days off during the less busy parts of the process. The peaks could also be reduced or removed by adjusting the detail of the production schedule or adopting flexible use of operators from other parts of the facility.

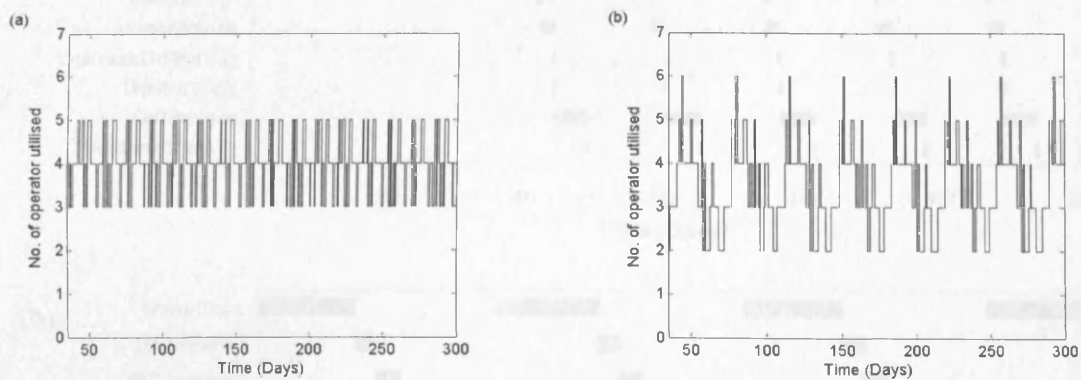


Figure 6.3. Utilisation of production operators for the (a) fed-batch and (b) perfusion mode of operation. The computed average utilisation of operators was 3.4 and 3.6 in the fed-batch and perfusion systems respectively.

The utilisation of USP and DSP equipment for successive batches is plotted in the Gantt charts in Figures 6.4a and b for the fed-batch and perfusion option respectively. The horizontal bars represent the utilisation time of the equipment. The equipment can be in use for either the product-manufacture or equipment-preparation steps. The seed train fermentation is scheduled such that there is minimum lead time between the completion of one batch and the initialisation of the next batch. The two manufacturing strategies possess different USP equipment utilisation characteristics. The figures indicate that there are more seeding batches in fed-batch as compared to the perfusion option. The shorter culture cycle in fed-batch necessitates more upstream seeding batches, hence the USP equipment is more fully

utilised in the fed-batch option. The affinity chromatography has a higher rate of utilisation in the perfusion mode due to the need for the daily harvest of liquor to be loaded onto the column. The remaining DSP equipment in both options possess similar utilisation characteristics.

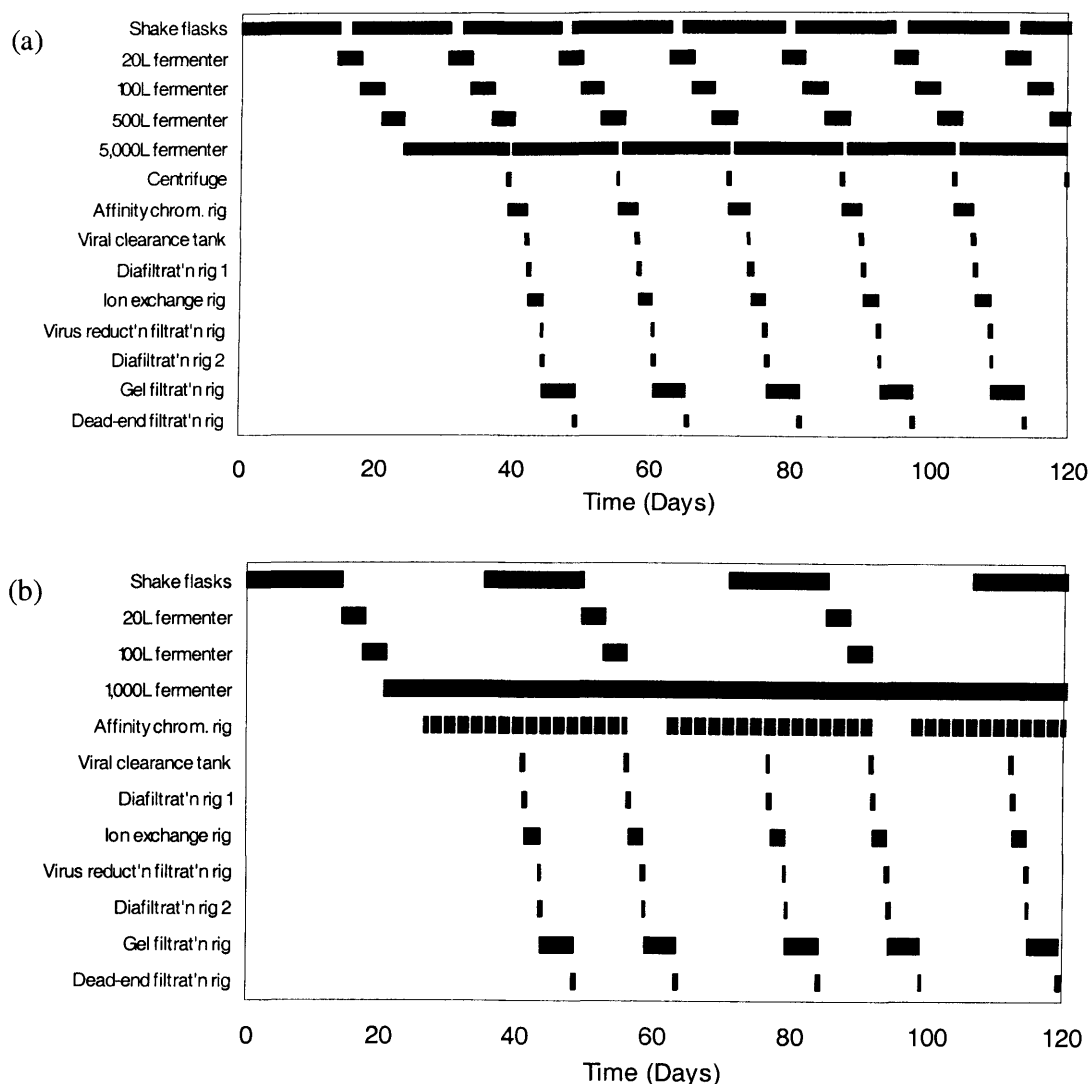


Figure 6.4. Gantt charts: Utilisation of equipment for (a) fed-batch and (b) perfusion mode of operation.

6.3.2. Economic Evaluation

The economic feasibility of the fed-batch and perfusion processes is summarised in Table 6.3. The perfusion values are relative to the fed-batch case. As expected, the use of fed-batch culture translates into a higher capital burden. The tool predicted that the perfusion option offers a significant reduction (42%) in the fixed capital expenditure relative to the fed-batch case due to the need for a smaller seed fermentation train and lower overall fermentation volume. Hence, the perfusion plant benefits from lower initial investment costs and seems attractive for a start-up company with potentially tight cash reserves. Comparison of the annual COG/g shows the two systems to be very similar, although the perfusion option offers a slight edge with a 3% reduction in the COG/g as compared to the fed-batch. The NPV of the perfusion strategy increases by 12% relative to the fed-batch process. The IRR of both cases was also computed. This value corresponded to 36% and 52% in the case of fed-batch and perfusion respectively. The perfusion option seems more economically feasible because of its higher NPV and IRR.

Table 6.3. Simulation results for key economic parameters. The perfusion values are relative to the fed-batch case.

Output parameters	Fed-batch	% change in perfusion relative to fed-batch
Fixed capital investment	\$24 million	-42%
Cost of goods per gram, COG/g	\$530/g	-3%
Net Present Value, NPV	\$57 million	+12%
Internal rate of return, IRR	36%	+16%

Figure 6.5 illustrates the cumulative cash flow of the two manufacturing options assuming a construction period of 3 years and a subsequent plant life of 10 years. The intersection of the curve with the x-axis indicates the payback time. The fed-batch option would take a longer time (5.4 years) to recover the original investment as opposed to the perfusion option (4.6 years). This can be attributed to the initial higher up-front investments required in the fed-batch option.

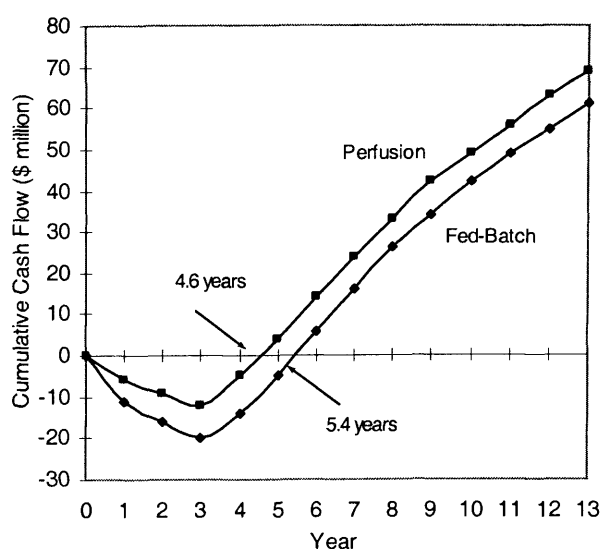


Figure 6.5. The diagram illustrates the cumulative cash flow of the process at a given year with a discount factor of 9%. The intersection of the curve with the x-axis indicates the payback time of the project.

It is possible to view the operating costs on a category basis (Figures 6.6a-c). Figure 6.6a shows the total COG/g broken down into direct (variable) costs such as the materials, utilities, staff and indirect (fixed) overheads such as the capital charge, tax, insurance and maintenance. The costs are relative to the fed-batch case. Although the total annual COG/g is similar for both options, the ratio of direct to indirect costs varies in each case. It is apparent that the COG/g in both the fed-batch and perfusion processes is dominated by the direct costs, which represent 62% and 76% of the total costs respectively. Although the fed-batch plant has lower direct costs as compared to the perfusion culture, the larger equipment scale contributes to higher indirect costs, thus increasing the overall costs. The capital charge is computed at an annual compound interest rate of 10% on the fixed capital investment over an operating plant life of 10 years. On this basis, it was expected that the perfusion plant would have a lower annual capital charge. The simulation results predicted a 10% decrease in the annual capital charge relative to the fed-batch case. Since the other fixed overheads costs (local tax, insurance, maintenance and general utilities) are proportional to the capital investment required, which is higher in the fed-batch case, these costs are significantly higher at about 14% of the total COG/g in the fed-batch option and decrease to about 9% in the perfusion system.

Figure 6.6b shows the annual direct cost outputs categorised under fermentation, purification and ancillary task headings. Again the costs are relative to the fed-batch case. Comparing these direct costs revealed a 21% increase in fermentation costs for perfusion culture due to the higher levels of usage of media and associated labour over the longer fermentation cycle. Examining the costs of the ancillary tasks (equipment-preparation and regulatory compliance activities) indicates that they represent 60% of the total direct costs in the fed-batch process, which falls to 42% in the perfusion process. The ancillary operation costs are higher under fed-batch operation due to the increased amount of resources required to clean and sterilise a larger production fermenter, as well as the higher frequency of CIP and SIP steps required with more bioreactor runs per year.

The annual direct COG/g can be further broken down to view the operational cost distributions of individual unit operations in the process flow. This reflects the cost of the direct materials, utilities and labour allocated to each manufacturing activity, thus providing the capability to view where the resource costs are concentrated. Figure 6.6c shows the annual direct costs for each of the manufacturing tasks for both the fed-batch and perfusion processes. In the fed-batch process, the CIP step is the most resource intensive and the most expensive task. The CIP costs include the direct costs incurred in the utilisation of labour and WFI, CIP buffer. This highlights the cost burden of cleaning operations in the fed-batch operation. In the perfusion process, the production fermentation contributes the most to the direct costs, followed by the CIP activities. The high fermentation production costs are mainly due to the higher usage of costly pre-made media in disposable bags over the extended perfusion culture cycle. This result implies that the process development might usefully seek ways to cut down the costs of media perhaps by in-house preparation rather than using ready-made media in disposable bags. Affinity chromatography tasks in both systems contribute to the direct cost but to a relatively less significant level. The remaining unit operations each make a minor contribution to the total direct COG/g.

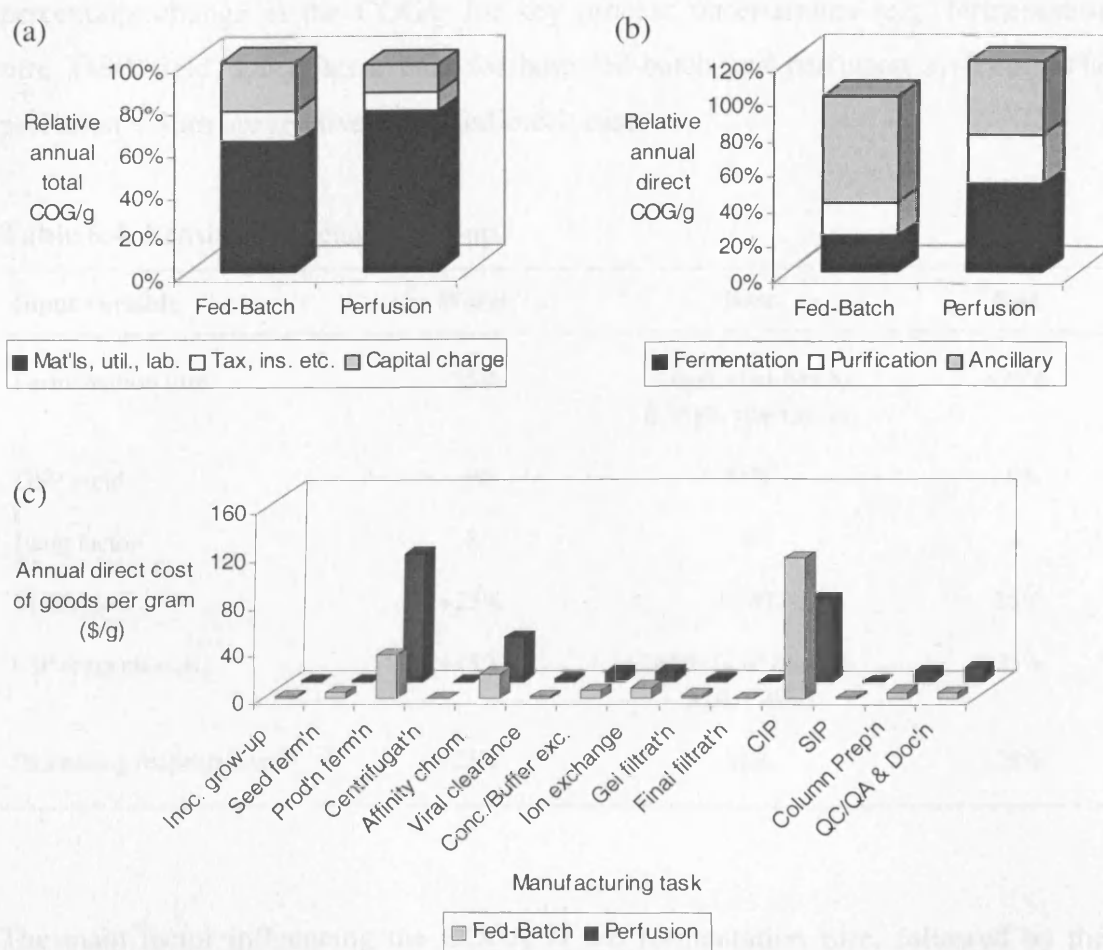


Figure 6.6. Distribution of production cost showing breakdowns for the (a) relative total annual expenses, (b) relative annual direct COG/g and (c) annual direct COG/g on a manufacturing task category. The perfusion values in (a) and (b) are relative to the fed-batch option.

6.4. Sensitivity Analysis

The range of values for the key input uncertainties in the process (Table 6.4) was determined from literature sources and through discussions with industrial experts. These values were selected to be a reflection of likely fluctuations occurring within similar batches for a single product. A sensitivity analysis was carried out for each variable to investigate the impact of variations in the initial assumptions on the output parameters. Such sensitivity analysis could help to determine the feasibility and attractiveness of the fed-batch and perfusion systems when individual financial and technical parameters in the process vary. The results in Figures 6.7a-f depict the

percentage change in the COG/g for key process uncertainties (e.g. fermentation titre, DSP yield, Lang factor etc.) for both fed-batch and perfusion systems. The perfusion values are relative to the fed-batch case.

Table 6.4. Sensitivity scenarios set-up.

Input variable	Worst	Base	Best
Fermentation titre	-25%	1.0g/L (fed-batch) 0.35g/L (perfusion)	+25%
DSP yield	-5%	55%	+5%
Lang factor	8	6	4
Media cost	+25%	\$20/L	-25%
CIP reagents cost	+25%	\$5/L (CIP buffer) \$1/L (WFI)	-25%
Processing reagents cost	+25%	\$5/L	-25%

The main factor influencing the COG/g is the fermentation titre, followed by the DSP yield. As indicated in Figure 6.7a, a $\pm 25\%$ change in fermentation titre represents a $\pm 17\%$ change in the annual COG/g in the fed-batch process. In perfusion, a 25% increase in the fermentation titre corresponds to a 19% drop in the COG/g relative to the fed-batch.

Since both manufacturing options were designed to have similar overall recovery yields and generate the same quantity of product annually, the gradients of the curves indicate that the COG/g of both strategies have the same degree of sensitivity to a change in the DSP yield (Figure 6.7b).

As indicated in Figure 6.7c, the COG/g in the fed-batch system is less susceptible to changes in media cost than under the perfusion mode of operation. A 25% reduction in media cost lowers the COG/g by 11% in the perfusion system as compared to a 2% decrease in the fed-batch process. The COG/g value of the perfusion system is relative to the fed-batch case. This is attributed to the fact that the media cost is a major contributor to the COG/g in the perfusion system. A point of intersection

occurs at a 10% increase in the cost of media (\$22/L), where both modes of operation have the same COG/g values. As the cost of media increases beyond \$22/L, the lower sensitivity of the fed-batch mode to this variable results in this mode of operation being more economically attractive than perfusion.

The CIP reagents cost has a relatively significant effect on the COG/g in both culture modes (Figure 6.7d). The CIP reagents included the CIP buffer and WFI, which are used for cleaning equipment (e.g. fermenters, chromatography skids). This reflects the large usage of buffer for cleaning steps. A 25% increase in the cost of CIP reagent leads to increases in the annual COG/g by about 7% and 2% in the fed-batch and perfusion systems respectively. Again, the perfusion value is relative to the fed-batch case. In the best scenario, given a 25% reduction in the cost of CIP reagents, both the fed-batch and perfusion options have the same annual COG/g. The perfusion strategy seems more favourable than fed-batch as the CIP reagents cost increases from the best case.

The indirect costs of the COG/g are proportional to the Lang factor and the total equipment purchase cost. Since the fed-batch system has higher indirect costs, the COG/g is fairly sensitive to the Lang factor assumed (Figure 6.7e). The perfusion process is marginally less sensitive.

Figure 6.7f indicates that the cost of processing reagents contribute to a relatively less significant change to the COG/g in the fed-batch option. With a 25% increase in the reagents cost, the fed-batch option is comparable to the perfusion culture on the basis of COG/g.

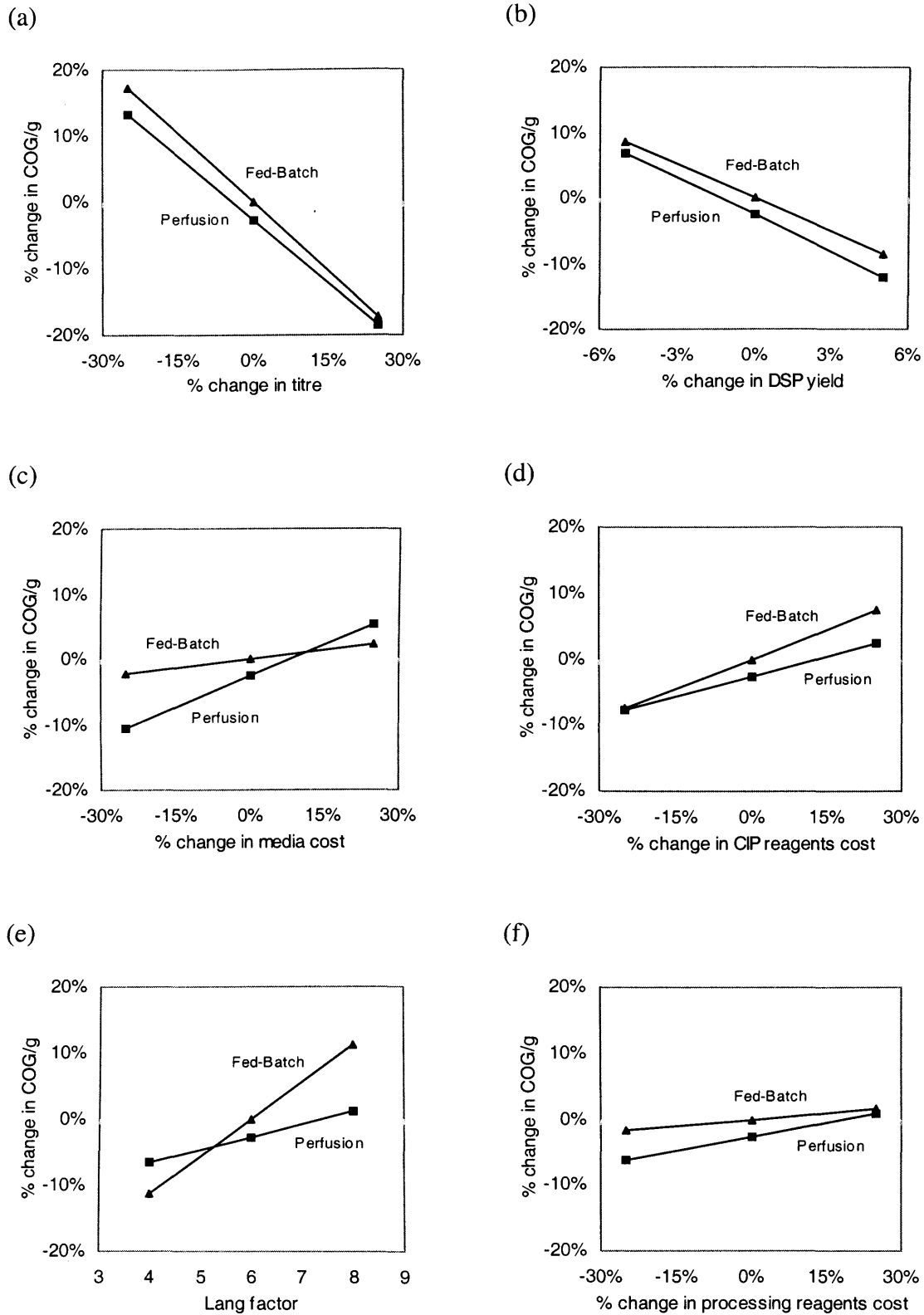


Figure 6.7. Sensitivity plots for (a) fermentation titre, (b) DSP yield, (c) media cost, (d) CIP reagents cost (e) Lang factor and (f) processing reagents cost. The values are relative to the fed-batch case.

6.5. Decision-Making using Probability Information

Sensitivity analysis was employed in the previous section to determine the behaviour of the COG/g performance measure to $\pm x\%$ changes in each uncertain factor and hence determine the stability of the base case. The analysis had considered uncertainties such as the fermentation titre, DSP yield and media cost. The variables that have the greatest impact on the performance measure were the fermentation titre and DSP yield. Risk factors such as batch contamination and filter fouling, which may affect the performance metrics, could not be captured by the sensitivity analysis method. Therefore, in this section, both the decision tree and Monte Carlo simulation techniques were employed to incorporate such uncertainties in order to evaluate the potential risk associated with each manufacturing option.

The construction of a fully expanded tree to take into account uncertainty in the fermentation titre, DSP yield, contamination and filter fouling would require a large number of possible outcomes to be considered and could be a time-consuming process. Hence, the fermentation titre and DSP yield were given average values. The effect of the range of such uncertainties could be investigated one at a time via sensitivity analysis using the decision tree framework. However, such an approach is not efficient. A Monte Carlo simulation method could be used to capture the degree of variability in the key influencing factors. The simulation results from each method can be interpreted in several ways to aid the decision-making process. The performance measures may show conflicting outcomes and the company may devise a strategy to indicate their preferences.

6.5.1. Decision Tree Analysis

6.5.1.1. Decision tree set-up

The key risk factors were the possibilities of contamination in both modes of operation and equipment failure due to filter fouling in perfusion culture. To take these factors into account, contamination probabilities, denoted as P_{FB_c} and P_{PF_c} , of 1% and 10% were incorporated in fed-batch and perfusion options respectively. The higher probability of contamination (10%) reflected the increased chance of a bioreactor run being contaminated in perfusion culture. In addition, the probability

of filter clogging, P_{PF_f} , (10%) was added to reflect the risk of equipment failure in perfusion culture. These values were determined through discussions with industrial experts (e.g. Francis, R., Protherics Plc., London, UK; Hoglund, E., Lonza Biologics, Portsmouth, USA). In reality, the risk of contamination and equipment failure could occur randomly during the fermentation process. For the decision tree analysis, the day on which the risk factors occurred were chosen to reflect likely events that could happen. The main purpose was to demonstrate the use of the decision tree framework to carry out risk analysis. It was assumed that the point of contamination occurred on Day 15 in both processes. In fed-batch, the entire broth in a bioreactor run would be lost. In perfusion, the daily pooling process results in the continual retrieval of the broth in a bioreactor run until contamination occurs. The mechanical failure of the filter was assumed to occur on Day 20 in the perfusion option. Upon the detection of contamination or filter clogging, the culture was then terminated and a new batch initiated. The average recoverable fermentation titres were 1g/L and 350mg/L for the fed-batch and perfusion systems respectively while the average DSP yield was 55% for both systems.

The different probable scenarios were envisaged and illustrated using a decision tree (Figure 6.8). The decision tree was set-up using an Excel spreadsheet. Each branch has an associated probability of occurrence. The *Choose* function, nC_k , in probability mathematics is employed to determine the number of total possible outcomes and the subsequent computation of the probability of each path. The index n equals the total scenarios to choose from and k equals the number of scenarios to be chosen. For example, 9C_1 (which equals 9) in the fed-batch option computes the number of possible outcomes where one out of nine bioreactors is contaminated. In the fed-batch option, the possible scenarios were the situations where none of the nine bioreactor runs were contaminated, followed by one bioreactor run being contaminated and so on. The software tool was used to run each scenario independently and to obtain the risk-adjusted performance metrics (i.e. annual output, COG/g and NPV). Simulation was carried out for subsequent scenarios, where the number of contaminated bioreactor runs was incremented by one, till the weighted value of the path became insignificant. The expected value of a given output parameter was calculated by multiplying the risk-adjusted value with the

probability in each path and then adding the weighted values of all the paths for each manufacturing option. As shown in Figure 6.8, the expected value for the NPV of each manufacturing strategy was indicated in a box.

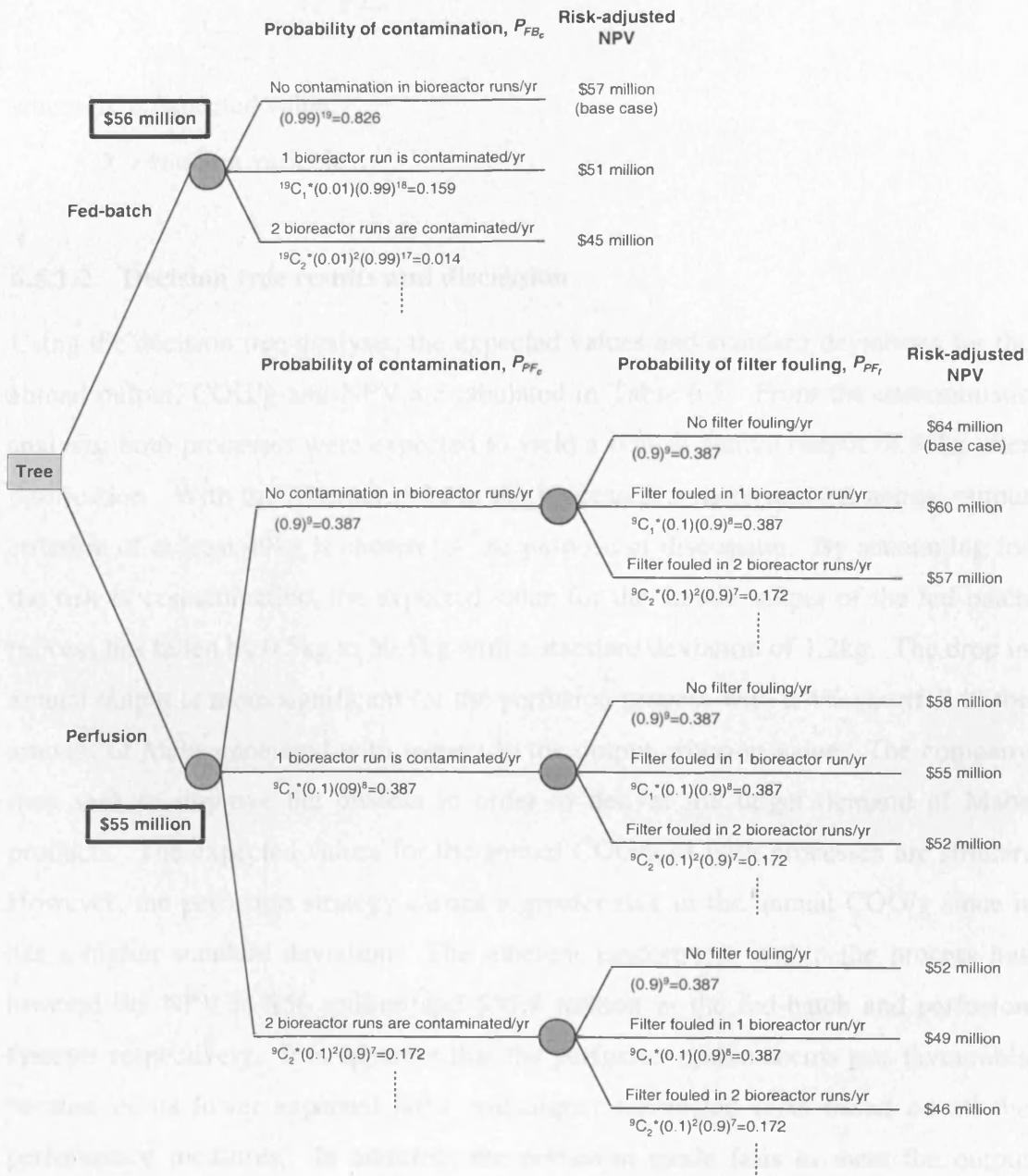


Figure 6.8. Decision tree with different probabilistic scenarios. Each branch has an associated probability of occurrence. The expected value for the NPV of each manufacturing strategy is indicated in the box.

Besides the expected values, the standard deviation of each performance measure was also computed. The equation for the standard deviation, σ , can be derived from statistical textbooks and is given by

$$\sigma = \sqrt{\sum X^2 P(X) - \mu^2} \quad (6.1)$$

where μ = expected value,

X = random variable.

6.5.1.2. Decision tree results and discussion

Using the decision tree analysis, the expected values and standard deviations for the annual output, COG/g and NPV are tabulated in Table 6.5. From the deterministic analysis, both processes were expected to yield a typical annual output of 51kg after purification. With the inherent risks in the processes, a more rational annual output criterion of at least 49kg is chosen for the purpose of discussion. By accounting for the risk of contamination, the expected value for the annual output of the fed-batch process has fallen by 0.5kg to 50.5kg with a standard deviation of 1.2kg. The drop in annual output is more significant for the perfusion process with a 4% shortfall in the amount of Mabs generated with respect to the output criterion value. The company may seek to improve the process in order to deliver the target demand of Mabs products. The expected values for the annual COG/g of both processes are similar. However, the perfusion strategy carries a greater risk in the annual COG/g since it has a higher standard deviation. The inherent randomness within the process has lowered the NPV to \$56 million and \$55.4 million in the fed-batch and perfusion systems respectively. It is apparent that the perfusion option seems less favourable because of its lower expected NPV and higher associated risks based on all the performance measures. In addition, the perfusion mode fails to meet the output criterion by a considerable amount (4% loss) and is therefore rendered non-feasible.

Table 6.5. Decision tree analysis: Expected values and standard deviations for the key performance measures.

Performance metric	Statistic	Value	
		Fed-Batch	Perfusion
Annual output (kg)	Expected value	50.5	47.0
	Standard deviation	1.2	4.0
Cost of goods per gram (\$/g)	Expected value	540	540
	Standard deviation	10	40
Net present value (\$ million)	Expected value	56.0	55.4
	Standard deviation	2.6	7.2

The primary benefit of the decision tree is that it provides an illustration of the decision-making process. Such a method emphasises the two competing alternative choices and identifies possible outcomes and their probabilities of occurrence. The rule for interpreting the decision tree is to select the path which either maximises value or minimises cost. For example, based on the decision tree analysis, the company would probably opt for the fed-batch option, which yields a higher expected NPV value. The decision tree facilitates the calculation of revised probabilities of occurrence and the subsequent computation of the expected values. As an illustration, the probabilities of the risk factors were varied and contour plots were generated to determine the feasible operational regions for the perfusion option.

Figures 6.9a and b illustrate the regions where percentage change in the annual output and NPV of the perfusion option are plotted as a function of the contamination and fouling probabilities, P_{PF_c} and P_{PF_f} . The NPV values are relative to that of the fed-batch option (\$56 million) with $P_{FB_c} = 1\%$. The annual output is relative to the annual criterion of 49kg. As illustrated in Figure 6.9a, any operation within the white area would result in a negative NPV relative to the fed-batch. The region below the white area indicates the feasible operational domains where the perfusion is economically more attractive than the fed-batch process in terms of NPV. However, in order to meet the annual output criterion of 49kg, the shaded

region above the solid line (Figure 6.9b) is not feasible. For example, although operating at point 'A', where $P_{PF_c} = 10\%$, and $P_{PF_f} = 8\%$, can achieve a higher NPV relative to fed-batch, it has an expected production mean of less than 49kg and hence is unacceptable. On the other hand, operating at point 'B' can meet the output criterion and yield a higher NPV than the fed-batch option. If the process was believed to be operating with a P_{PF_c} of 10%, then the maximum tolerable P_{PF_f} would be 5% (point 'C').

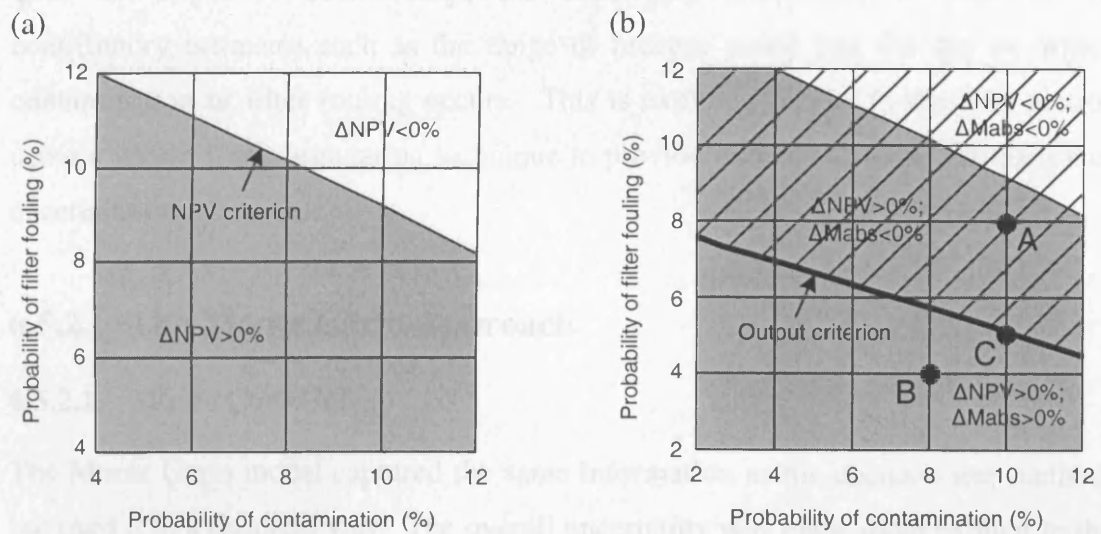


Figure 6.9. The surface contour plot illustrates the percentage change in (a) NPV and (b) both the annual output and NPV of the perfusion culture as a function of P_{PF_c} and P_{PF_f} . $\Delta Mabs$ represents the percentage change in the annual output relative to the output criterion of 49kg and ΔNPV is the percentage change in NPV relative to that of the fed-batch culture.

6.5.1.3. Conclusions

The decision tree method is both simple and effective. The tool facilitated the setting up and simulation of the probable scenarios in the decision-tree. However, decision trees do have some weakness associated with their use. The actual construction of a decision tree can be a time-consuming process for each project. In this particular

case study, the fermentation titre and DSP yield were given average values to simplify the assessment of the decision tree approach. If the variations in these factors were taken into account during the analysis, the tree would become very complicated to account for all the possible combinations of titres, DSP yields, possibilities of contamination and filter fouling. This would require several additional simulation runs for all the possible scenarios and it would become inefficient to carry out the decision tree method. Also, decision trees are not ideal for parallel events that could happen. An example is the simultaneous occurrence of both contamination and filter clogging in a single perfusion run. Such an approach does not explicitly acknowledge the underlying uncertainty in each of the contributory estimates such as the range of process yields and the day on which contamination or filter fouling occurs. This is explored further in the next section using a Monte Carlo simulation technique to provide a more complete estimation of uncertainties.

6.5.2. The Monte Carlo Approach

6.5.2.1. Monte Carlo set-up

The Monte Carlo model captured the same information as the decision tree method, but used it in a different way. The overall uncertainty was made more explicit in the Monte Carlo simulation, where the model quantified the uncertainty by allowing the ranges for the various activities to be estimated. Table 6.6 specifies the key input risk parameters considered, together with their ranges of values. The fermentation titre was subjected to a variation of $\pm 25\%$ and the DSP yield ranged between $\pm 5\%$ of the base value for both the fed-batch and perfusion systems. These two variables were given a discrete Normal distribution. P_{FB_c} and P_{PF_c} values of 1% and 10% were incorporated in the fed-batch and perfusion systems respectively. A P_{PF_f} value of 10% was added to the perfusion culture. The *Input Random Number* block provided in Extend was used to generate random numbers according to the distributions specified in Table 6.6. When executed, the model randomly generated a particular day between Day 1 and Day 30 on which a contamination or filter clogging occurs according to a negatively-skewed triangular distribution (i.e. contamination and filter fouling are more likely to occur towards the end of the fermentation run). Such a

distribution was sensible since it represented the increased chances of failure over time. The Monte Carlo simulation technique was used to characterise the variability in the performance measures due to uncertainties in titre, DSP yield and risk factors. The following discussion highlights possible analyses that can be performed to interpret the data obtained from the Monte Carlo simulations and to compare the relative merits of the two options.

Table 6.6. Monte Carlo simulation set-up: Input risk parameters and their discrete probability distributions.

Variable	Units	Fed-batch		Perfusion	
		Value	Probability	Value	Probability
Fermentation titre	g/L	0.75	0.1	0.26	0.1
		0.88	0.2	0.31	0.2
		1.0	0.4	0.35	0.4
		1.13	0.2	0.39	0.2
		1.25	0.1	0.44	0.1
DSP yield	%	50	0.1	50	0.1
		53	0.2	53	0.2
		55	0.4	55	0.4
		58	0.2	58	0.2
		60	0.1	60	0.1
Perfusion run contaminated?	-	Yes	0.01	Yes	0.1
		No	0.99	No	0.9
Filter in perfusion run clogged?	-	-	-	Yes	0.1
		-	-	No	0.9

6.5.2.2. Monte Carlo simulation results and discussion

The Monte Carlo simulation technique enables the range of possible outcomes to be generated by running the model many times and creates various statistical summaries featuring the designated outputs. Figures 6.10a-c depict the comparison of the probability frequency distributions for the performance metrics of both the fed-batch

and perfusion systems. In addition to the computation of expected values and standard deviations of key performance measures, other statistics such as the likelihood of not achieving or exceeding a certain value are tabulated. Table 6.7 summarises the key results of the Monte Carlo simulation for the fed-batch and perfusion systems and the ranking of the alternatives.

Comparing the distribution graphs in Figures 6.10a-c reveals that the fed-batch option presents a less risky alternative because the probability distributions of outcomes are much narrower. The actual standard deviations of each performance measure are given in Table 6.7. The perfusion strategy has higher associated risks based on all performance measures. The frequency distributions of all the performance metrics in the fed-batch option are nearly symmetrical.

Figure 6.10a indicates that the frequency distribution curve for the annual output of the perfusion option is bimodal. The two modes occur at 46kg and 50kg, with frequency probabilities of 12% and 18% respectively. The minor peak at 46kg represents a 5kg drop in the annual output as compared to the deterministic case. This corresponds to an average output of one perfusion run being lost. The loss in such a situation can be due to the occurrence of either contamination or filter fouling, or even both occurring simultaneously. The simulation results suggest the output will usually be around 50kg. However, there would also be a relatively high occurrence of an output of 46kg. From the data presented in Table 6.7, the uncertainties in process titres and yields, together with the risk factors, have shifted the annual production means to 49.5kg and 48.0kg in the fed-batch and perfusion options respectively. The probability of not achieving at least 49kg of Mabs annually is 21% in the fed-batch option. The perfusion strategy has a higher risk (42%) of not meeting the output criterion.

There exists a wide range of possible outcomes for the annual COG/g in both options (Figure 6.10b). The annual COG/g distribution graph for the perfusion option is skewed positively. While the majority of the COG/g values are clustered toward the lower end of the scale, the risk factors in the process have resulted in a relatively high score of COG/g values at the upper end of the scale. Although the modal COG/g is \$520/g, the mean value has been shifted to \$540/g. As indicated in Table

6.7, both processes have comparable expected COG/g value but the perfusion option possesses a greater risk. In the fed-batch case, there is a 64% chance that the projected cost would exceed the baseline COG/g value derived from the deterministic analysis. This probability is higher (80%) in the perfusion case. These outputs draw attention to the need to reduce the variance in the cost of goods, which is especially true for the perfusion option.

For the NPV analysis, a threshold criterion of at least \$56 million may be chosen to decide if the particular manufacturing strategy is feasible. Figure 6.10c indicates that the uncertainties in the process have shifted the curve towards the lower end of the scale. Interestingly the perfusion option is ranked first based on its expected NPV but second based on its standard deviation (Table 6.7). The probability of not achieving a NPV of at least \$56 million is higher (52%) in the fed-batch option compared to 32% in the perfusion option. However, the results generated by the tool indicate that even though the perfusion option has a higher NPV, it has expected production mean below the 49kg target and is therefore not feasible. The fed-batch emerges as the more favourable option with a higher likely output and lowest associated risks based on all performance measures.

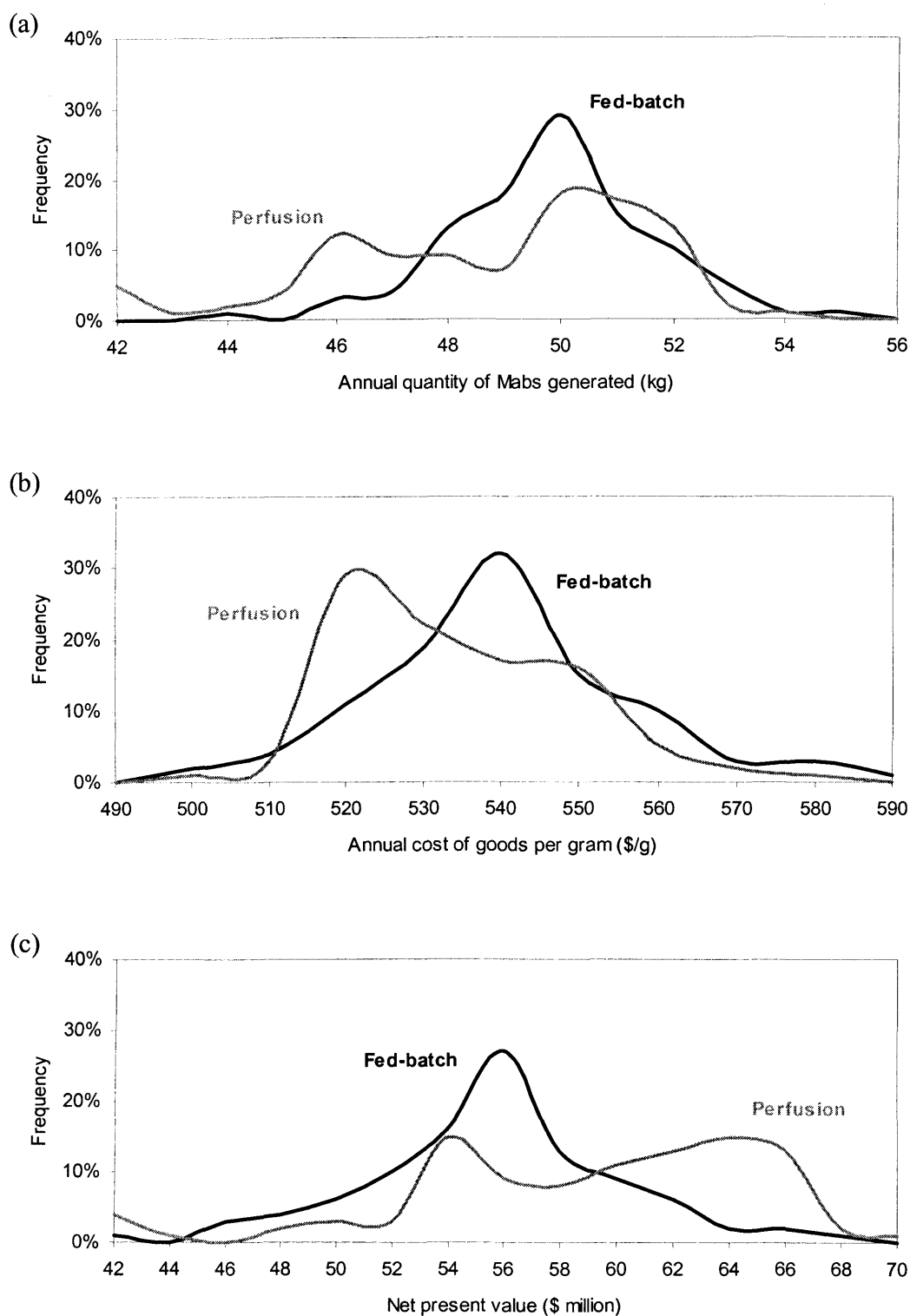


Figure 6.10. Frequency distributions for the (a) annual quantity of Mabs generated, (b) annual COG/g and (c) NPV of both the fed-batch and perfusion systems.

Table 6.7. Summary of key results for the Monte Carlo analysis of fed-batch and perfusion systems.

Performance metric	Statistics	Value		Ranking order (best first)
		FB*	PF*	
Annual output (kg)	Expected value	49.5	48.0	FB, PF
	Standard deviation	1.9	4.5	FB, PF
	P(Failing to yield at least 49kg of Mabs annually)	21%	42%	FB, PF
Cost of goods per gram (\$/g)	Expected value	540	540	-
	Standard deviation	20	70	FB, PF
	P(Exceeding baseline COG/g value obtained by deterministic analysis)	64%	80%	FB, PF
Net present value (\$ million)	Expected value	55.4	57.0	PF, FB
	Standard deviation	5.0	10.5	FB, PF
	P(Failing to achieve an NPV value of at least \$56 million)	52%	32%	PF, FB

* FB/PF = Fed-batch/Perfusion

It is possible that the assumed chances of contamination and filter clogging in perfusion cultures are too pessimistic (Personal communication: Francis, R., Protherics Plc., London, UK; Hoglund, E., Lonza Biologics, Portsmouth, USA). Accordingly, two further scenarios 'a' ($P_{PF_c} = 5\%$; $P_{PF_f} = 5\%$) and 'b' ($P_{PF_c} = 3\%$; $P_{PF_f} = 3\%$) were selected by varying both the probabilities of contamination and filter fouling in the perfusion option. The Monte Carlo simulation was used to run

each scenario and determine the key output measures and their associated risks. Figure 6.11 is the bubble plot corresponding to the different scenarios. The various scenarios are plotted on an axis of the expected annual quantity of Mabs generated versus the probability of contamination. The size of the bubble is proportional to the reward/risk ratio. This ratio measures the expected NPV over the standard deviation in each scenario. The rejected scenarios are marked with a cross.

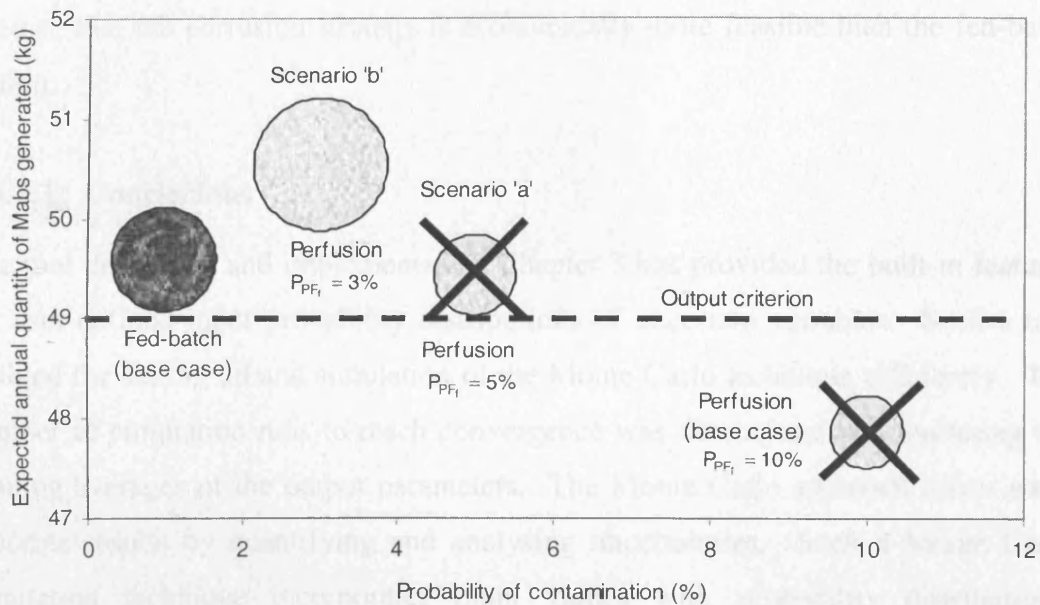


Figure 6.11. Bubble plot for the different scenarios. The x-axis corresponds to the expected annual quantity of Mabs generated and the y-axis represents the probability of contamination. The size of the bubble is proportional to the reward/risk ratio. This ratio measures the expected NPV over the standard deviation in each scenario.

It is intuitive that scenarios with a low probability of contamination, high likely output and high reward/risk ratios are preferred. The prioritisation of options based on bubble plots depends upon the risk attitude of the management. A risk-averse management might prefer scenarios with high returns and low risks, i.e. a high reward/risk ratio. The annual output criterion was set to be at least 49kg and the scenarios were ranked based on the reward/risk ratio. The perfusion base case, where $P_{PF_c} = 10\%$ and $P_{PF_i} = 10\%$, is rejected since it fails the output criterion of

49kg. Based on the bubble plot, for scenario 'a' where $P_{PF_c} = 5\%$ and $P_{PF_f} = 5\%$, although the perfusion option has expected production mean above 49kg, the reward/risk ratio is smaller than the fed-batch option. This is evident from the relative size of the bubble to the fed-batch process. This option is therefore rejected. Examining the data in Figure 6.11 indicates that by reducing both P_{PF_c} and P_{PF_f} to 3% (scenario 'b'), the perfusion option has a higher reward/risk ratio compared to the fed-batch strategy. Hence, considerable efforts are required to reduce both the probabilities of contamination and filter fouling in order to improve the reward/risk ratio so that the perfusion strategy is economically more feasible than the fed-batch option.

6.5.2.3. Conclusions

The tool developed and implemented in Chapter 3 has provided the built-in features for user-defined input probability distributions of uncertain variables. Such a tool enabled the setting up and simulation of the Monte Carlo technique efficiently. The number of simulation runs to reach convergence was determined by monitoring the running averages of the output parameters. The Monte Carlo approach offers more accurate results by quantifying and analysing uncertainties. Such a Monte Carlo simulation technique incorporates input values with probability distributions, generates the range of possible outcomes and enables the frequency probability distributions of performance metrics to be plotted. Besides the expected values of output parameters, it allows the determination of useful statistics such as the likelihood of failing to meet or exceeding a threshold value. However, unlike decision trees, Monte Carlo simulations do not explicitly recommend a course of action or make decision. Although the tool provided the ability to enable the input probability distributions of uncertain factors to be specified, the Monte Carlo approach is very time-consuming in terms of computation. Each simulation carried out in the decision-support tool required a substantial amount of time to complete a Monte Carlo run.

6.5.3. Decision Tree versus Monte Carlo

The previous two sections have presented the use of the decision-support tool to evaluate the uncertainties inherent in bioprocesses using the decision tree analysis and the Monte Carlo simulation method. The tool had provided a suitable platform for these two different risk techniques to be carried out. Both techniques were efficiently implemented in the tool to obtain the simulation results. However, there were some weaknesses associated with the use of the tool to perform the risk assessment. Since the decision tree analysis required each possible scenario to be simulated independently to determine the outcome, the application of such a technique was unsuitable when there were a large number of scenarios to be considered. Hence, the application of the decision tree technique was efficient by limiting the number of possible scenarios. The main disadvantage of using the tool for the Monte Carlo approach was its relatively high computational time required to reach convergence. The computational aspect of the tool could be enhanced by improving the coding efficiency; this would be discussed in the future work in the next chapter.

6.6. Overall Chapter Conclusions

A computer-aided approach to the evaluation of the process economics of fed-batch and perfusion culture for the commercial production of Mabs has been presented via a case study. The deterministic analysis shows that whilst on the basis of COG/g, there is little difference between the two routes, the perfusion option benefits from lower capital investment and higher projected NPV. A sensitivity study indicated that the annual COG/g of both processes is most sensitive to the fermentation titre, followed by the DSP yield. The decision tree and Monte Carlo simulation techniques were employed to account for the possibilities of contamination and equipment failure. The information generated by the simulation studies can help to assess the feasibility of alternatives and provide a more holistic approach to the evaluation of manufacturing strategies. The decision tree analysis demonstrated that the perfusion option is inferior to the fed-batch process because of its lower likely output, lower expected NPV and higher risks based on all the performance measures.

The viability of the perfusion process could be improved by reducing the rate of contamination and filter fouling. The feasible operational domains were illustrated in a surface contour plot where the perfusion option meets the output criterion and has a higher projected NPV than fed-batch. The Monte Carlo simulation technique demonstrated that although the perfusion option has a higher expected NPV, it fails the output criterion and hence is non-feasible. The critical failure rate at which the perfusion option is more favourable than fed-batch was identified.

This chapter highlights the benefits of using the decision tree technique to provide a graphical illustration of the decision-making process and as an aid in the evaluation of manufacturing alternatives based on expected values. Although the decision tree method provides a simple and efficient approach for risk analysis, it does not explicitly quantify the overall uncertainty. The Monte Carlo simulation method was therefore applied to provide a more accurate estimation of process uncertainties. Such a technique provides a way of incorporating probability distributions of input parameters to identify the range of possible outcomes and generate frequency distributions for performance measures. The main disadvantage of the Monte Carlo approach is its relatively high computational time required to reach convergence.

CHAPTER 7

Conclusions and Future Work

7.1. Introduction

With growing demand on the clinical pipeline, efficient manufacturing is emerging as a key issue in the biotech industry. The performance of manufacturing has a great influence on the timely delivery of drugs to the market and affects the financial health of a company. Global competition is driving the need to improve manufacturing efficiency through the need to achieve the following business and process objectives: accelerated time-to-market, improved process yields, maximised resource utilisation and reduced cost of goods. The need for computer-aided design tools to act in support of making the best business decisions relating to biomanufacture is becoming increasingly critical. Simulation tools can prove invaluable in the understanding of manufacturing systems, characterisation of unit operations and selection of process options; all of which contribute to better decision-making in the context of the business and process needs shaping the bioprocess sector. This final chapter summarises the contributions made in this thesis in exploring the possible development and deployment of a decision-support tool to aid the assessment of manufacturing alternatives from economic as well as process perspectives. It also recommends future work that can be carried out to address further issues in the biomanufacturing industry by the research.

7.2. Overall Conclusions

An introductory background to the biopharmaceutical drug development and manufacturing pathway has been presented in Chapter 1. The recent surge in demand for antibodies has increased the need for large-scale efficient production of these biochemical entities. Early process development is necessary to enhance manufacturing operations in order to streamline timescales whilst containing costs. The application of computer-aided design tools is critical to aid process design and development of biochemical processes. The need for a bioprocess simulator that captures both the business and process perspectives of biopharmaceutical manufacturing is becoming increasingly important. However, process performance is affected by the need to comply with regulatory practices and to cope with randomness inherent in the system. Existing simulators often lack the capabilities to reflect regulatory conformation and incorporate uncertainties. These factors provided part of the impetus and motivation for this research, which examines the capacity of a bioprocess simulator to provide a business-process interface, model regulatory-compliance activities and incorporate risks in processes subject to uncertainty.

In Chapter 2, the industrial production of Mabs for large-scale applications is described as this provided the basis for the case studies in Chapters 5 and 6. There exist various expression techniques and cell culture methods for the commercial production of protein-derived products. A review of published literature indicated that mammalian cell culture is the most common technology for the expression of such recombinant proteins. The industry has converged on using stirred tank bioreactors as the standard technology for large-scale production of antibodies. Fed-batch and perfusion cultures are the two dominant modes of operation used for such mammalian-based processes. Typical industrial issues concerning the manufacture of antibodies, which provide the foundation for the case studies in this thesis, are highlighted. An investigation of the commercial production methods for Mabs enabled generic mammalian-based processes to be identified that were subsequently used as the basis for the unit operations included in the tool domain. Recent technologies used in the development of antibodies were discussed so as to assess future trends in antibody production.

The development and implementation of a prototype tool, *BioPharmKit*, to model bioprocess operations in the manufacture of monoclonal antibodies is illustrated in Chapter 3. The main objective was to formulate a decision-support tool to compute cost measures, simulate resource handling, perform mass balance calculations and incorporate risks in order to facilitate the transparency and ease of decision-making in the biopharmaceutical industry. The conceptual framework seeks to integrate the technical, operational and economic aspects of biopharmaceutical manufacture. The hierarchical task-oriented approach employed in this work was adapted from previous work at University College London (UCL) and has been extended to include regulatory compliance activities. Such an approach enables the rapid modelling of information at different levels of details. The tool architecture also permits the explicit modelling of ancillary tasks such as equipment cleaning and regulatory activities, so as to estimate more accurately operative measures such as costs and resource utilisation. The graphical illustration and dynamic simulation of the process offers a comprehensive perspective of the modelling system. The economic feasibility and effectiveness of manufacturing options can be evaluated by examining key performance metrics such as the cost of goods, capital investment and net present value (NPV). Incorporating risk analysis permits the evaluation of potential risks associated with different manufacturing strategies and enhances the quality of decision-making within a company.

The usefulness of the simulation tool developed in this thesis is explored in Chapter 4. The tool can be employed to model a new plant during the early design stage. In addition, the tool can act as a platform to simulate optimal operating scenarios using existing plant capacity. It can also describe a range of case studies and help to address the typical problems facing the industry. Effective use of the simulation outcomes can contribute to improved process development, more efficient use of resources and result in worthwhile comparisons of manufacturing alternatives. The application of the prototype tool for accessing the impact of manufacturing alternatives on technical and financial performance metrics was demonstrated via industrial-related case studies in the subsequent chapters.

A typical issue in perfusion culture is how often to pool the fermentation broth in order to utilise fully resources while reducing operating costs. In the case study

described in Chapter 5, which assessed the most appropriate pooling strategies in perfusion mode using mammalian cell culture, the modelling tool indicates how manufacturing options affect the demands on resources and manufacturing costs. A deterministic case was initially carried out and validated by industrial experts to ensure the outputs were computed correctly. A sensitivity analysis was then performed to determine the impact of individual variables on key output parameters. The effects of fluctuating titres, process yields and risk of contamination were analysed using the Monte Carlo simulation technique in order to reflect the inherent variability of process parameters during the operation of a biopharmaceutical plant. The stochastic simulation forecasts a range of possible outcomes, determines the likelihood that the key performance metrics could exceed a critical threshold, and computes the expected performance of the model in order to facilitate process analysis. Possible scenarios regarding the stability of product cell line and downstream purification scale were analysed to gain further insight into relevant events and enhance the decision-making.

Fed-batch and perfusion cultures are capable of achieving large-scale production of antibodies. Although the fed-batch culture has predominated, there has been increasing use of perfusion culture in recent years due to the high productivity achieved in such culture system. The use of the decision-support tool to compare the economic feasibility of both culture modes was investigated in Chapter 6. In the deterministic analysis, the values of the key economic and process output parameters were determined. To take into account the inherent variability of process variables, the key uncertainties in the system were identified and a sensitivity analysis was then performed to investigate the impact on output parameters. Another objective of this chapter was to compare risk analysis methods, such as the decision tree and Monte Carlo simulation techniques, to account for risk factors with known probabilistic characteristics. An illustration of how to determine the probabilistic cases and compute the expected output values using a decision tree was provided. The decision tree analysis provided a simple and efficient approach to select between manufacturing alternatives based on the expected values of performance metrics. The limitation of the decision tree analysis was found to be the inability to explicitly acknowledge the underlying uncertainty in the contributory estimates and to consider parallel events that could happen. A Monte Carlo analysis was then performed to

· provide a more accurate estimation of the overall uncertainty. Such a technique incorporates input parameters with probability distributions, generates frequency distributions for each designated output and computes various statistical summaries featuring the outputs. However, unlike decision trees, the Monte Carlo technique is more time-consuming in terms of computation and does not explicitly make a decision.

The work carried out in this thesis presents the design and implementation of a decision-support tool that links both the process and business aspects of biopharmaceutical manufacture. The tool, *BioPharmkit*, has customised built-in features specific for bioprocesses including costing functions to determine plant cost, operating costs and profitability of manufacturing options, mass balance models to calculate process data, risk parameters to carry out risk assessment and a graphical interface to build model flowsheets. In addition, the tool offers the ability to create newer unit operation models. Key disadvantages of the approach are that significant effort was required to customise existing unit operations models. This is because *BioPharmkit* does not have the availability of bioprocess models and sufficient data required to accommodate more rigorous and sophisticated models. Despite the limitations mentioned, the potential rewards of such a bioprocess simulation tool are significant. The benefits of the simulation tool for strategic decision-making have been demonstrated through application to industrial-related case studies. It is envisaged that such a tool might be used during the early stage of process development, which can lead to faster time-to-market, more efficient use of resources and enhanced the economic performance of a company.

7.3. Thesis Highlights

The novelty of this thesis is the development and implementation of a decision-support tool, *BioPharmKit*, with features specific to biomanufacture. The implementation of such a tool was based on the production of Mabs expressed in mammalian cells. Generic processes for the production of marketed Mabs have been used to provide the foundation for the unit operations included in the tool domain. The functionalities of the tool have been illustrated via application to two

industrially-related case studies. The following section highlights the major contributions contained in this thesis.

- *Integration of business and process aspects of biomanufacture*

The economic and process aspects of biomanufacturing are often quantitatively unconnected. The work in this thesis has linked both the business and process perspectives of the biopharmaceutical manufacturing industry in order to aid the decision-making process. The business aspects deal with the fixed capital investment, direct and indirect costs, cost of goods and NPV while the process aspects consider issues such as mass balance, resource utilisation profiles and annual output. The tool has included these features so as to model each of the business and process perspectives of biomanufacturing and to provide a more complete evaluation of manufacturing alternatives.

- *Model the impact of regulatory-compliance activities*

The decision-making process is often complicated by the need to comply with regulatory issues. Traditional simulation tools often omit the modelling of such regulatory activities in the biomanufacturing environment. The addition of these activities with process flowsheet models is crucial to investigate the impact of such tasks on the process output parameters. The hierarchical, task-oriented tool implemented in this thesis provides the ability to model ancillary tasks explicitly. The regulatory-compliance tasks such as QC/QA and batch documentation have been implemented in the decision-support tool to enable the impact of such activities on the performance of the process to be investigated.

- *Customised unit operation specific for perfusion culture*

The operation of perfusion culture involves a continuous feed of media into the bioreactor while a continuous amount of spent culture is withdrawn. One complexity of the perfusion mode is the need for a retention device (e.g. spin filter) to retain the cells in the bioreactor. The tool is constructed in a modular way to provide the ability to extend and customise so as to include newer unit operations. Such implementation enables different culture modes to be evaluated based on profitability and process indices.

- *Provide a user-friendly interface*

The decision-support tool developed in this thesis is user-friendly and allows the user to employ such a tool with great speed. The graphical user interface feature provides a clear visualisation of the simulation flowsheet. The dynamic animation of the tool enables the user to view the execution of tasks during the simulation process. The “drag” and “drop” feature of the decision-support tool simplifies the interaction between the user and the tool. Dialog boxes have been cloned from the Extend coding blocks to enable efficient communication.

- *Incorporate risk analysis*

Commercially available simulators often lack the capability to represent uncertainties in biomanufacture. Such randomness would have an impact on the decision-making process and hence the need to incorporate risk factors in simulation software is becoming increasingly important. The ability to carry out risk analysis would allow the risks associated with different manufacturing options to be evaluated and hence minimise the risk of incorrect conclusions as a result of the variability of the process parameters. The decision-support tool in this thesis has accommodated the randomness stemming from fluctuating fermentation titres, process yields, the possibilities of contamination and filter clogging. Such uncertainties are represented as Normal discrete distributions. Both the decision-tree and Monte Carlo simulation techniques, which are typical tools used for risk analysis, have been employed to evaluate the impact of uncertainties on key performance metrics.

7.4. Future Work

Biotechnology simulation is a rapidly advancing tool for biopharmaceutical manufacture. It allows the rapid assessment of a large number of options to be made when development goes beyond the level of initial laboratory studies. The decision-support tool developed in this thesis could evolve into a powerful tool for assisting decision-making. The framework established lays a strong foundation for exploring further work that can be used to address key issues in the industry. There are clearly many potential areas available for improving and extending the functionalities of the tool. The future work proposed in this section would be essential to enhance further

the capabilities and robustness of the tool to aid the process of decision-making. Several examples are pointed out and discussed below.

- *Improving coding efficiency of the tool*

As discussed in Chapter 6, the simulation of each Monte Carlo run required a substantial amount of time. The computational speed of the tool could be improved by coding the mass balance calculations in Excel. This would greatly reduce the number of blocks required to configure each unit operation and less computational time would be needed to initialise and process these blocks during the simulation. In addition, simple calculations of the process yields for each unit operation would be sufficient instead of using the rigorous mass balance calculations adopted in this thesis.

- *Customisation of new unit operations*

The construction of the tool in a modular and extensible manner has facilitated the development of applications and allowed the developer to extend new features with ease. Such customisation should enable the impact of new manufacturing technologies to be assessed based on running costs and manufacturing performance. The ability of the tool to build and customise new unit operations is demonstrated in a parallel study carried out at UCL (Mustafa *et al.*, 2004). In this work, techniques such as expanded bed adsorption (EBA) chromatography are explored and developed in the software package. A case study is set up to compare the process and business benefits of a conventional process route employing packed chromatography beds and an alternative that uses EBA. The next phase of the study aims to investigate the potential feasibility of an EBA retrofit within a conventional packed-bed based process.

- *Modelling of explicit manufacturing tasks*

In order to simplify the tool implementation process, material preparation steps were omitted from the modelling process. For instance, media and buffer are assumed to arrive pre-made in disposable bags. In future work, these activities could be modelled explicitly to achieve a more complete analysis. The incorporation of such preparation steps is essential to an examination of the impact on the resultant cost of goods and demand on resources, such as operator and materials. The modelling of

these ancillary tasks could be achieved by communication with the finance and manufacturing departments of the companies so as to determine the relevant costs and typical raw materials demand.

- *Integration with drug development pathway*

In addition to the work presented in this thesis, parallel work (Rajapakse *et al.*, 2004) has focused on simulating the activities for portfolio management during biopharmaceutical drug development from drug discovery to market launch. Linking such a framework with the model of biopharmaceutical manufacture, as described in this thesis, would allow the impact of manufacturing decisions to be evaluated based on development timescales and costs, as well as manufacturing performance such as resource utilisation and cost of goods.

- *Linkage to a sophisticated database*

The data for populating the model was obtained from an existing UCL database and verified through discussions with industrial experts and biopharmaceutical vendors. Microsoft Excel has been employed as the database providing a transparent interface to the Extend platform. A more sophisticated database (e.g. SQL, Oracle) could enhance the performance of the simulation tool. These databases offer more powerful capacities for organizing, managing and retrieving data. This needs to incorporate manufacturing data for different production processes for the various types of products.

The future work discussed in this section would be essential to capture pertinent features relating to biomanufacture and to enhance the capabilities the tool. In addition to improving the features of the decision-support tool, the tool could be used to investigate other industrially-related case studies. The case studies presented in this thesis have illustrated the application of the decision-support tool to assess the viability of manufacturing options in terms of process and economic aspects. The tool could be used to investigate novel manufacturing techniques so as to gain insights into real manufacturing situations. For instance, production of antibody-based proteins in transgenic goats, chickens and various plant varieties has been recently developed to achieve high yields at a potential lower cost of goods and

capital investment. A detailed analysis of transgenic production is essential to provide a clear perception of these new process options.

Further simulation studies using the tool could be applied to the ultra scale-down project currently undertaken at UCL (Titchener-Hooker *et al.*, 2001). The ultra scale-down mimics methodology allows the rapid prediction of the best way in which to produce complex biopharmaceuticals during the early stages of biopharmaceutical development pathway and where only small volumes of material are available for study. Such methods alone are inadequate to predict completely the production performance. The decision-support tool could be employed to supplement the scale-down and ultra scale-down strategies by allowing a capacity to ask “what if” questions and to link the different scales of operation.

In conclusion, the tool developed in this thesis has generated new areas for further investigation. The development of more sophisticated simulation capabilities would increase the functionalities of the tool and enable more complex and rigorous modelling to be accomplished. The application of industrial-related case studies provides insights into real manufacturing situations and aids the evaluation of process scenarios. The simulation tool possesses great potential for growth as a computer-based decisional system to address strategic needs for the successful commercialisation of biopharmaceuticals. Such bioprocess simulators could become significant engineering tools for process design and development over the next decade.

References

Allen, D. H. *Economic Evaluation of Projects – A Guide*, 3rd edn.; Institute of Chemical Engineers; Hertford, England: Stephen Austin & Sons Ltd., 1991.

Andersen, D. C.; Krummen, L. Recombinant Protein Expression for Therapeutic Applications. *Curr. Opin. Biotechnol.*, **2002**, *13*, 117-123.

Aranha, H.; Forbes, S. Viral Clearance Strategies for Biopharmaceutical Safety. *Pharm. Technol.*, **2001**, *Jun*, 26-40.

Bailey, J. E.; Ollis, D. F. BioProcess Economics. In *Biochemical Engineering Fundamentals*, 2nd edn.; New York; London: McGraw-Hill, 1986; Chapter 12.

Baker, S. J. & Wheelwright, S. M. Financially Based Modelling of Recovery Process Alternatives. *BioProcess Int.*, **2004**, *May*, 42-54.

Banik, G. G.; Heath, C. A. Partial and Total Cell Retention in a Filtration-Based Homogeneous Perfusion Reactor. *Biotechnol. Prog.*, **1995**, *11*, 584-588.

Banks, J. Principles of Simulation. In *Handbook of Simulation: Principles, Methodology, Advances, Applications, and Practice*; Banks, J. (Ed.); Wiley: Canada, 1998; Chapter 1.

Bartley, A.; MacLeod, A. J. A Comparative Study of Monoclonal Antibody Yield using Batch, Continuous or Perfusion Suspension Culture Techniques. In *Animal Cell Technology: Developments, Processes and Products*; Spier, R. E., Griffiths, J. B., Macdonald, C. (Eds.); Butterworth-Heinemann: Oxford, UK, 1992; pp 376-378.

Bebbington, C. R.; Renner, G.; Thomson, S.; King, D.; Abrams, D.; Yarranton, G. T. High-Level Expression of a Recombinant Antibody from Myeloma Cells using a Glutamine Synthetase Gene as an Amplifiable Selectable Marker, *Bio/Technol.*, **1992**, *10*, 169-175.

Berthold, W. Cost Elements for Supply during Antibody Production Development. Presented at Production and Economics of Biopharmaceuticals – Economics of Capacity Expansion and Creative Ways to Bridge the Capacity Crunch. San Diego, CA, IBC, Nov 18-23, 2002.

Bibila, T. A.; Robinson, D. K. In Pursuit of the Optimal Fed-Batch Process for Monoclonal Antibody Production. *Biotechnol. Prog.*, **1995**, *11*, 1-13.

Birch, J. R. Suspension Culture, Animal Cells. In *Encyclopaedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation*; Flickinger, M. C., Drew, S. W. (Eds); Wiley: New York; Chichester, 1999; Vol 5.

Birch, J. R.; Froud, S. J. Mammalian Cell Culture Systems for Recombinant Protein Production. *Biologicals*, **1994**, *22*, 127-133.

Blau, G.; Mehta, B.; Bose, S.; Pekny, J.; Sinclair, G.; Keunker, K.; Bunch, P. Risk Management in the Development of New Products in Highly Regulated Industries. *Comput. Chem. Eng.*, **2000**, *24*, 659-664.

Brastow, W. C.; Rice, C. W. Planning Pharmaceutical Manufacturing Strategies in an Uncertain World. *BioProcess Int.*, **2003**, *Jun*, 46-55.

Brown, M. E.; Renner, G.; Field, R. P.; Hassell, T. Process Development for the Production of Recombinant Antibodies using the Glutamine Synthetase (GS) System. *Cytotechnol.*, **1992**, *9*, 231-236.

Byrom, D. Role and Timing of Process Development for Biopharmaceutical Manufacture. *Pharm. Technol. Eur.*, **2000**, *Mar*, *12*(3), 52-56.

Chadd, H. E.; Chamow, S. M. Therapeutic Anitbody Expression Technology. *Curr. Opin. Biotechnol.*, **2001**, *12*, 188-194.

Chow, S.-C. Pharmaceutical Validation and Process Controls in Drug Developments. *Drug Inf. J.*, **1997**, *31*, 1195-1201.

Chu, L.; Robinson, D. K. Industrial Choices for Protein Production by Large-Scale Cell Culture. *Curr. Opin. Biotechnol.*, **2001**, *12*, 180-187.

Clemento, A. New and Integrated Approaches to Successful Accelerated Drug Development. *Drug Inf. J.*, **1999**, *33*, 699-710.

Coates, E. R.; Kuhl, M. E. Using Simulation Software to Solve Engineering Economy Problems. *Comput. Ind. Eng.*, **2003**, *45*, 285-294.

Curling, J. The Cost of Chromatography. Presented at Production and Economics of Biopharmaceuticals. La Jolla, CA, IBC, Nov 13-15, 2000.

Dalm, C. Determining Viability in Perfusion Bioreactors. *Genet. Eng. News*, **2003**, *23*(16), 60, 62.

Datar, R. V.; Cartwright, T.; Rosen, C.-G. Process Economics of Animal Cell and Bacterial Fermentations: A Case Study Analysis of Tissue Plasminogen Activator. *Bio/Technol.*, **1993**, *11*, 349-356.

De Keyser, J.-L. High-Cell-Density Perfused Culture for Mabs. *Genet. Eng. News*, **2002**, *22*(10), 52.

Deo, Y. M.; Mahadevan, M. D.; Fuchs, R. Practical Considerations in Operation and Scale-up of Spin-filter Based Bioreactors for Monoclonal Antibody Production. *Biotechnol. Prog.*, **1996**, *12*, 57-64.

DePalma, A. Protein Products Push Large-Scale Cell Culture. *Genet. Eng. News*, **2002**, *22*(7), 58, 61.

DiMasi, J. A.; Hansen, R. W.; Grabowski, H. G. The Price of Innovation: New Estimates of Drug Development Costs. *J. Health Econ.*, **2003**, *22*, 151-185.

Doblhoff-Dier, O.; Bliem, R. Quality Control and Assurance from the Development to the Production of Biopharmaceuticals. *TIBTECH*, **1999**, *17*, 266-270.

Doctor, R. N.; Newton, D. P.; Pearson, A. Managing Uncertainty in Research and Development. *Technovation*, **2001**, *21*, 79-90.

Dove, A. Uncorking the Biomanufacturing Bottleneck. *Nat. Biotechnol.*, **2002**, *20*, 777-779.

Dynamic Chiropractic. Why Are U.S. Drug Prices So High?, **2003**, *21*(25).
www.chrioweb.com (accessed 2004)

Evans, L. B.; Field, R. P. Bioprocess Simulation: A New Tool for Process Development. *Chem. Eng. Aust.*, **1989**, *14*(4), 20-23.

Fahrner, R. L.; Blank, G. S.; Zapata, G. A. Expanded Bed Protein A Affinity Chromatography of a Recombinant Humanized Monoclonal Antibody: Process Development, Operation, and Comparison with a Packed Bed Method. *J. Biotechnol.*, **1999**, *75*, 273-280.

Farid, S. A Decision-Support Tool for Simulating the Process and Business Perspectives of Biopharmaceutical Manufacture. PhD thesis; UCL; 2001.

Farid, S.; Novais, J. L.; Karri, S.; Washbrook, J.; Titchener-Hooker, N. J. A Tool for Modelling Strategic Decisions in Cell Culture Manufacturing. *Biotechnol. Prog.*, **2000a**, *16*(5), 829-836.

Farid, S.; Washbrook, J.; Birch, J.; Titchener-Hooker, N. J. A Hierarchical Framework for Modelling Biopharmaceutical Manufacture to Address Process and Business Needs. In *Computer-Aided Chemical Engineering: ESCAPE-10*; S. Pierucci (Ed); Elsevier Science B.V.: Amsterdam, 2000b; 8, pp. 673-678.

Farid, S.; Washbrook, J.; Titchener-Hooker, N. J. Decision-Support Tool for Risk Analysis in Biopharmaceutical Manufacture. In *8th International Conference on Computer Applications in Biotechnology (CAB8): Modelling and Control of Biotechnological Processes*; Dochain, D., Perrier, M. (Eds); IFAC, EFB, 2001; pp. 167-171.

Fisher, M. P.; Pascucci, V. L. Regulatory Reflections Concerning the State of Biotechnology Progress, *Drug Inf. J.*, **1996**, *30*, 41-46.

Focus on Catalysts. Global Biotechnology Market Expected to Post Ten-fold Growth. **2003**(4), Apr, 2.

Foo, F.; Karri, S.; Davies, E.; Titchener-Hooker, N. J.; Dunhill, P. Biopharmaceutical Process Development: Part I, Information from the First Product Generation. *BioPharm Eur.*, **2001**, Jun, 58-64.

Furey, J. Scale-up of a Cell Culture Perfusion Process. *Genet. Eng. News*, **2002**, *22*(7), 62.

Garber, K. Biotech Industry Faces New Bottleneck. *Nat. Biotechnol.*, **2001**, *19*, 184-185.

Geigert, J. The Challenge of Managing Quality Control/Quality Assurance for Loosely Structured and Virtual Biopharmaceutical Companies. *Drug Inf. J.*, **1997**, *31*, 97-100.

Gerson, D. F.; Himes, V.; Hopfer, R.; Khandke, L.; Kohn, F.; Komotar, A.; Krumm, P.; Machulski, J.; Weisser, A.; Sciotto-Brown, S. Transfer of Processes from Development to Manufacturing. *Drug Inf. J.*, **1998**, *32*, 19-26.

Ginsberg, P. L.; Bhatia, S.; McMinn, R. L. The Road Ahead for Biologics Manufacturing. US Bancorp Piper Jaffray Equity Research, **2002**, 1-23.

Glaser, V. Biopharma Firms Must Mind Their QAs & QCs. *Genet. Eng. News*, **2002**, 22(8), 16, 18, 65.

González, Y.; Ibarra, N.; Gómez, H.; González, M.; Dorta, L.; Padilla, S.; Valdés, R. Expanded Bed Adsorption Processing of Mammalian Cell Culture Fluid: Comparison with Packed Bed Affinity Chromatography. *J. Chromatogr. B*, **2003**, 784, 183-187.

Gosse, M. E.; Manocchia, M. The First Biopharmaceuticals Approved in the United States: 1980-1994. *Drug Inf. J.*, **1996**, 30, 991-1001.

Grimster, S. Biopharmaceutical Manufacturing: Past, Present and Future. *Eur. Biopharm. Rev.*, **2003**, 88-92.

Gura, T. Magic Bullets Hit the Target. *Nat.*, **2002**, 417, 584-586.

Happel, J.; Jordan, D. G. *Chemical Process Economics*, 2nd edn.; New York: Marcel Dekker, Inc., 1975; Chapter 3.

Hesse, F.; Wagner, R. Developments and Improvements in the Manufacturing of Human Therapeutics with Mammalian Cell Cultures. *TIBTECH*, **2000**, 18, 173-180.

Hjorth, R. Expanded-bed Adsorption in Industrial Bioprocessing: Recent Developments. *TIBTECH*, **1997**, 15, 230-235.

Hodge, G. Economics of Disposable Process Components for the Manufacture of Monoclonal Antibodies. Presented at Production and Economics of Biopharmaceuticals – Alternative Technologies for Speed to Market and Cost Reduction. San Diego, CA, IBC, Nov 18-23, 2002.

Holmer, A. F. New Biotechnology Medicines in Development, 2002, Pharmaceutical Research and Manufacturers of America (PHRMA), <http://www.phrma.org>. (accessed 2003)

Hood, E. E.; Woodard, S. L.; Horn, M. E. Monoclonal Antibody Manufacturing in Transgenic Plants – Myths and Realities. *Curr. Opin. Biotechnol.*, **2002**, *13*, 630-635.

Hoorn, P. V. Continuous Perfusion vs Batch Fermentation. Presented at Production and Economics of Biopharmaceuticals – Transcending the Limits of Manufacturing Medicines. La Jolla CA, IBC, Nov 13-15, 2000.

Houdebine, L.-M. Antibody Manufacture in Transgenic Animals and Comparisons with Other Systems. *Curr. Opin. Biotechnol.*, **2002**, *13*, 625-629.

Hu, W.-S.; Aunins, J. G. Large-scale Mammalian Cell Culture. *Curr. Opin. Biotechnol.*, **1997**, *8*, 148-153.

Humphrey, A. Some Issues in Biotechnology Commercialisation. *Technol. Soc.*, **1996**, *18*(3), 321-332.

Iding, K.; Lütkemeyer, D. Fraune, E.; Gerlach, K.; Lehmann, J. Influence of Alternations in Culture Condition and Changes in Perfusion Parameters on the Retention Performance of a 20µm Spin-filter during a Perfusion Cultivation of a Recombinant CHO Cell Line in Pilot Scale. *Cytotechnol.*, **2000**, *34*, 141-150.

Imagine That, Inc. Extend User's Guide V.5.0, 2000.

Jayme, D. W.; Smith, S. R. Media Formulation Options and Manufacturing Process Controls to Safeguard Against Introduction of Animal Origin Contaminants in Animal Cell Culture. *Cytotechnol.*, **2000**, *33*, 27-36.

Kadouri, A.; Spier, R. E. Some Myths and Messages Concerning the Batch and Continuous Culture of Animal Cells. *Cytotechnol.*, **1997**, *24*, 89-98.

Karri, S.; Davies, E.; Washbrook, J.; Titchener-Hooker, N. J. Biopharmaceutical Process Development: Part III, A Framework to Assist Decision Making. *Biopharm Eur.*, **2001**, *Sep*, 76-82.

Kelly, B. D. Biochemical Engineering Bioprocessing of Therapeutic Proteins. *Curr. Opin. Biotechnol.*, **2001**, *12*, 173-174.

King, D. J. Production of Monoclonal Antibodies. In *Applications and Engineering of Monoclonal Antibodies*; Taylor & Francis: Philadelphia, 1998; Chapter 5.

Lamble, N. Therapeutic Proteins: Strategic Market Analysis and Forecasts to 2010. Datamonitor plc., 2000. www.datamonitor.com (accessed 2002)

Lang, H. J. Simplified Approach to Preliminary Cost Estimates. *Chem. Eng.*, **1948**, *55*, 112-113.

Larrick, J. W.; Thomas, D. W. Producing Proteins in Transgenic Plants and Animals. *Curr. Opin. Biotechnol.*, **2001**, *12*, 411-418.

Law, A. M.; Kelton, W. D. Basic Simulation Modeling. In *Simulation Modeling & Analysis*, 2nd edn.; McGraw-Hill, 1991; Chapter 1.

Lawlis, B. A Worldwide Shortage of Biomanufacturing Capacity for Monoclonal Antibodies. Presented at Production and Economics of Biopharmaceuticals – Transcending the Limits of Manufacturing Medicines. La Jolla, CA, IBC, Nov 13-15, 2000.

Lias, R.; Fogerty, S. The Squeeze in Contract Biomanufacturing. *Pharm. Technol. Eur.*, **2002**, *Jun*, 31-34.

Macmillan, J. D.; Velez, D. Miller, L.; Reuveny, S. Monoclonal Antibody Production in Stirred Reactors. In *Large Scale Cell Culture Technology*; Lydersen, B. K., (Ed.); Hanser Publishers: Munich, 1987; pp. 22-58.

Mahadevan, M. D. Bioreactor Process Selection for Large-Scale Manufacture of Monoclonal Antibodies – Tradeoffs and Recommendations. *Bioprocessing J.*, **2003**, 25-31.

Marks, L.; Power, E. Using Technology to Address Recruitment Issues in the Clinical Trial Process. *TIBTECH*, **2002**, 20(3), 105-109.

Martin, N.; Brennan, A.; Denome, L.; Shaevitz, J. High Productivity in Mammalian Cell Culture. *Bio/Technol.*, **1989**, 5, 838-840.

Matejtschuk, P.; Baker, R. M.; Chapman, G. E. Purification and Characterization of Monoclonal Antibodies. In *Bioseparation and Bioprocessing Volume II: Processing, Quality and Characterization, Economics, Safety and Hygiene*; Subramanian, G. (Ed); Wiley-VCH: Weinheim, 1998; Chapter 8.

McNamara, L. Where is the Blockbuster Market Heading? An Analysis of the Current and Future Blockbuster Market Until 2008. Industry Dynamics, 2002. www.samedanltd.com (accessed 2003)

Mercille, S.; Johnson, M.; Lanthier, S.; Kamen, A. A.; Massie, B. Understanding Factors that Limit the Productivity of Suspension-Based Perfusion Cultures Operated at High Medium Renewal Rates. *Biotechnol. Bioeng.*, **2000**, 67(4), 435-450.

Molowa, D. T.; Shenouda, M. S.; Meyers, A. P.; Tublin, P. W.; Fein, A. S. The State of Biologics Manufacturing: Part 2. JP Morgan Securities Inc., Equity Research, **2002**, 1-16.

Monge, M.; Hammarberg, B. Disposable Technology for Biomanufacturing: Scaling-up within a Modular Facility. Presented at Scaling Up of Biopharmaceutical Products. The Grand, Amsterdam, IBC, Jan 26-27, 2004.

Moran, E. B.; McGowan, S. T.; McGuire, J. M.; Frankland, J. E.; Oyebade, I. A.; Waller, W.; Archer, L. C.; Morris, L. O.; Pandya, J.; Nathan, S. R.; Smith, L.; Cadette, M. L.; Michalowski, J. T. A Systematic Approach to the Validation Process Control Parameters for Monoclonal Antibody Production in Fed-Batch Culture of a Murine Myeloma. *Biotechnol. Bioeng.*, **2000**, 69(3), 242-255.

Morrow, K. J. BioPharm Manufacturing Capacities. *Genet. Eng News*, **2001**, 21(21), 1, 60, 61, 85.

Morrow, K. J. Industrial-Scale Antibody Production Strategies. *Genet. Eng News*, **2002**, 22(17), 8-71.

Mustafa, M. A., Washbrook, J., Lim, A. C., Zhou, Y., Titchener-Hooker, N. J., Morton, P.; Berezenko, S.; Farid, S. A Software Tool to Assist Business-Process Decision-Making in the Biopharmaceutical Industry. *Biotechnol. Prog.*, **2004**, 20(4), 1096-1102.

Myers, S. C.; Howe, C. D. A Life-Cycle Financial Model of Pharmaceutical R&D. Program on the Pharmaceutical Industry, Massachusetts Institute of Technology, 1997.

Nichols, N. A. Scientific Management at Merck: An Interview with CFO Judy Lewent. *Harvard Bus. Rev.*, **1994**, 89-99.

Nicholson, I. J.; Latham, P. When to “Make or Buy” Means “Make or Break”. *Bio/Technol.*, **1994**, 12, 473-477.

Norris, P. Seamless Process Development: Enhancing Speed to Market and Reducing Costs. *Pharm. Visions*. **2001**, 36-39.

Novais, J. L.; Titchener-Hooker, N. J.; Hoare, M. Economic Comparison Between Conventional and Disposable-Based Technology for the Production of Biopharmaceuticals. *Biotechnol. Bioeng.*, **2001**, 75(2), 143-153.

Ohashi, R.; Singh, V.; Hamel, J.-F. P. Perfusion Culture in Disposable Bioreactors. *Genet. Eng. News*, **2001**, 21(7), 40, 78.

Onigman, P. Transgenic Production Update. Presented at Production and Economics of Biopharmaceuticals – Transcending the Limits of Manufacturing Medicines. La Jolla, CA, IBC, Nov 13-15, 2000.

Pandey, M. Investment Decisions in Pharmaceutical R&D Projects. *Drug Discov Today*, **2003**, 8(21), 968-971.

Peter, M. S.; Timmerhaus, K. D. Plant Design and Economics for Chemical Engineers; McGraw-Hill: New York; London, 1991.

Petrides, D. P. BioPro Designer: An Advanced Computing Environment for Modelling and Design of Integrated Biochemical Processes. *Comput. Chem. Eng.*, **1994**, 18 S, S621-S625.

Petrides, D. P.; Cooney, C. L.; Evans, L. B.; Field, R. P.; Snoswell, M. Bioprocess Simulation: An Integrated Approach to Process Development. *Comput. Chem. Eng.*, **1989**, 13(4/5), 553-561.

Petrides, D. P.; Calandranis, J.; Cooney, C. Computer-Aided Design Techniques for Integrated Biochemical Processes. *Genet. Eng. News*, **1995a**, 10, 28, 29, 35.

Petrides, D. P.; Sapidou, E.; Calandranis, J. Computer-Aided Process Analysis and Economic Evaluation for Biosynthetic Human Insulin Production – A Case Study. *Biotechnol. Bioeng.*, **1995b**, 48(5), pp. 529-541.

Petrides, D. P.; Calandranis, J.; Cooney, C. Bioprocess Optimization via CAPD and Simulation for Product Commercialization. *Genet. Eng. News*, **1996**, 24, 28, 40.

Pharmaceutical Industry Profile, Chapter 3, pp. 27-39, 1999.

Pisano, G. P.; Wheelwright, S. C. High-Tech R&D, The Logic of High-Tech R&D. *Harvard Bus. Rev.*, **1995**, 94-105.

Pollock, D. P.; Kutzko, J. P.; Birck-Wilson, E.; Williams, J. L.; Echelard, Y.; Meade, H. M. Transgenic Milk as a Method for the Production of Recombinant Antibodies. *J. Immunol. Methods*, **1999**, 231, 147-157.

Raiffa, H. *Decision Analysis: Introductory Lectures on choices under Uncertainty*, London: Addison-Wesley, 1968.

Rajapakse, A., Titchener-Hooker, N. J., Farid, S. Modelling of the Biopharmaceutical Drug Development Pathway and Portfolio Management. In *Computer-Aided Chemical Engineering: ESCAPE-14*; Barbosa-Póvoa, A., Matos, H. (Eds); Elsevier Science B.V.: Lisbon, 2004; 18.

Ramsay, B. Lean Compliance in Manufacturing. *Pharm. Technol. Eur.*, **2001**, Oct, 62-68.

Reichert, J. M. New Biopharmaceuticals in the USA: Trends in Development and Marketing Approvals 1995-1999. *TIBTECH*, Sep **2000**, 18, 364-369.

Reichert, J. M. Monoclonal Antibodies in the Clinic. *Nat. Biotechnol.*, **2001**, Sep, 19, 819-822.

Reichert, J. M. Trends in Development and Approval Times for New Therapeutics in the United States. *Nat. Rev. Drug Discov.*, **2003**, 2, 695-702.

Reichert, J.; Pavlou, A. From the Analyst's Couch Monoclonal Antibodies Market. *Nat. Rev. Drug. Discov.*, **2004**, 3, 383-384.

Remer, D. S; Idrovo, J. H. Cost-Estimating Factors for Biopharmaceutical Process Equipment. *Pharm. Technol. Int.*, **1991**, Mar, 36-42.

Reuveny, S.; Velez, D.; Miller, L.; Macmillan, J. D. Comparison of Cell Propagation Methods for their Effect on Monoclonal Antibody Yield in Fermentors. *J. Immunol. Methods*, **1986**, 86, 61-69.

Rouf, S. A.; Moo-Young, M.; Scharer, J. M.; Douglas, P. L. Single versus Multiple Bioreactor Scale-up: Economy for High-value Products. *Biochem. Eng. J.*, **2000**, 6, 25-31.

Rouf, S. A.; Douglas, P. L.; Moo-Young, M.; Scharer, J. M. Computer Simulation for Large Scale Bioprocess Design. *Biochem. Eng. J.*, **2001a**, 8, 229-234.

Rouf, S. A.; Douglas, P. L., Moo-Young; M., Scharer, J. M. Economics of Fed-batch Operation: A Computer-aided Approach. *Bioprocess and Biosystems Eng.*, **2001b**, 24, 65-71.

Rudolph, N. S. Biopharmaceutical Production in Transgenic Livestock. *TIBTECH*, **1999**, 17, 367-374.

Savage, C. Scaleup and Bioproduction. *Genet. Eng. News*, **2000**, 1, 65, 80.

Schenerman, M. A.; Hope, J. N.; Kletke, C.; Singh, J. K., Kimura, R.; Tsao, E. I.; Folena-Wasserman, G. Comparability Testing of a Humanized Monoclonal Antibody (Synagis®) to Support Cell Line Stability, Process Validation, and Scale-Up for Manufacturing. *Biologicals*, **1999**, 27, 203-215.

Seaver, S. S. Lessons Learned from Working with Contract Manufacturers. *BioPharm*, **1995**, 8(2), 20-25.

Shanklin, T.; Roper, K.; Yegneswaran, P. K; Marten, M. R. Selection of Bioprocess Simulation Software for Industrial Applications. *Biotechnol. Bioeng.*, **2001**, 72(4), 483-489.

Sharpe, P.; Keelin, T. How SmithKline Beecham Makes Better Resource-Allocation Decisions. *Harvard Bus. Rev.*, **1998**, 45-57.

Sherman-Gold, R. Monoclonal Antibodies: The Evolution from '80s Magic Bullets to Mature, Mainstream Applications as Clinical Therapeutics. *Genet. Eng. News*, **1997**, 4, 35.

Sherwood, D. Biopharmaceutical Contract Manufacturing: An Overview. *Bioprocessing J.*, **2003**, 33-35.

Sinclair, A. Capacity Availability: Impact on Commercialisation Strategies. Screening 2-3, **2003**, GIT VERLAG GmbH & Co. KG, Darmstadt, 36-38. www.gitverlag.com/go/screening (assessed 2003)

Singh, V. Disposable Bioreactor for Cell Culture using Wave-Induced Agitation. *Cytotechnol.*, **1999**, 30, 149-158.

Sinnott, R. K. In *Coulson & Richardson's chemical engineering (Chemical engineering design)*; Pergamon Press: Oxford, 1993; Vol. 6.

Stewart, J. J.; Allison, P. N.; Johnson, R. S. Putting a Price on Biotechnology. *Nat. Biotechnol.*, **2001**, 19, 813-817.

Taylor, B. W. Decision Analysis and Game Theory. In *Introduction to Management Science*; Prentice-Hall, Inc.: New Jersey; 1999.

Titchener-Hooker, N. J.; Zhou, Y.; Hoare, M.; Dunnill, P. Biopharmaceutical Process Development: Part II, Methods of Reducing Development Time. *BioPharm Eur.*, **2001**, Sep, 68-74.

Tyler, J. E. Microencapsulation of Mammalian Cells. In *Large-scale Mammalian Cell Culture Technology*; Lubiniecki, A. S. (Ed.); Marcel Dekker, Inc.: New York, 1990; pp. 343-361.

Voisard, D.; Meuwly, F.; Ruffieux, P.-A.; Baer, G.; Kadouri, A. Potential of Cell Retention Techniques for Large-Scale High-Density Perfusion Culture of Suspended Mammalian Cells. *Biotechnol. Bioeng.*, **2003**, 82(7), 751-765.

Walsh, G. *Biopharmaceuticals: Biochemistry and Biotechnology*; Wiley: Chichester, 1998.

Walsh, G. Biopharmaceutical Benchmarks. *Nat. Biotechnol.*, **2000**, Aug, 18, 831-833.

Walsh, G.; Murphy, B. *Biopharmaceuticals, an Industrial Perspective*; Kluwer Academic Publishers, 2000; Chapter 1.

Walter, P. K. Cost and Capacity Comparison of Transgenics and Cell Culture Production Systems. Presented at Production and Economics of Biopharmaceuticals – Transcending the Limits of Manufacturing Medicines. La Jolla, CA, IBC, Nov 13-15, 2000.

Wechsler, J. Drug Shortages Reveal GMP Concerns. *Pharm. Technol. Eur.*, **2002**, Jun, 17-20.

Werner, R. G. Potential and Efficiency in the Biotechnical Process. *Pharm. Technol. Eur.*, **1994**, May, 20-28.

Wheelwright, S. M. *Protein Purification: Design and Scale up of Downstream Processing*. Hanser Publishers: Munich, Germany; 1991.

Woodside, S. M.; Bowen, B. D.; Piret, J. M. Mammalian Cell Retention Devices for Stirred Perfusion Bioreactors. *Cytotechnol.*, **1998**, 28, 163-175.

Xie, L.; Wang, D. I. C. High Cell Density and High Monoclonal Antibody Production through Medium Design and Rational Control in a Bioreactor. *Biotechnol. Bioeng.*, **1996**, 51(6), 725-729.

Yabannavar, V. M.; Singh, V.; Connelly, N. V. Mammalian Cell Retention in a Spinfilter Perfusion Bioreactor. *Biotechnol. Bioeng.*, **1992**, 40(8), 925-933.

Yabannavar, V. M.; Singh, V.; Connelly, N. V. Scaleup of Spinfilter Perfusion Bioreactor for Mammalian Cell Retention. *Biotechnol. Bioeng.*, **1994**, 43(2), 159-164.

Young, M. W.; Okita, W. B.; Brown, E. M.; Curling, J. M. Production of Biopharmaceutical Proteins in the Milk of Transgenic Dairy Animals. *BioPharm*, **1997**, 10(6), 34-38.

Young, M. W.; Meade, H.; Curling, J. M.; Ziomek, C. A.; Harvey, M. Production of Recombinant Antibodies in the Milk of Transgenic Animals. *Research in Immunol.*, **1998a**, 149(6), 609- 610.

Young, M. W.; Tiedemann, G.; Pepin, R. A.; Turner, J. D.; Okita, W. B.; Harvey, M.; Groet, S.; Curling, J. M. Transgenics Go Commercial. *BioPharm*, **1998b**, Jul.

Zhou, W.; Chen, C.-C.; Buckland, B.; Aunins, J. Fed-Batch Culture of Recombinant NSO Myeloma Cells with High Monoclonal Antibody Production. *Biotechnol. Bioeng.*, **1997**, 55(5), 783-792.

APPENDIX A

List of Publications

Refereed Papers

Lim, A. C.; Zhou, Y.; Washbrook, J.; Titchener-Hooker, N. J.; Farid, S. A Decision-Support Tool to Model the Impact of Regulatory Compliance Activities in the Biomanufacturing Industry. *Comput. Chem. Eng.*, **2004**, 28(5), 727-735.

Mustafa, M. A., Washbrook, J., Lim, A. C., Zhou, Y., Titchener-Hooker, N. J., Morton, P.; Berezenko, S.; Farid, S. A Software Tool to Assist Business-Process Decision-Making in the Biopharmaceutical Industry, *Biotechnol. Prog.*, **2004**, 20(4), 1096-1102.

Conference Paper

Lim, A. C.; Farid, S.; Washbrook, J.; Titchener-Hooker, N. J. A Tool for Modelling the Impact of Regulatory Compliance Activities on the Biomanufacturing Industry. In *Computer-Aided Chemical Engineering: ESCAPE-13*; Kraslawski, A., Turunen, I. (Eds); Elsevier Science B. V.: Lappeenranta, 2003; 14, pp. 1109-1114.

Oral Presentations

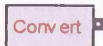
Lim, A. C.; Farid, S.; Washbrook, J.; Titchener-Hooker, N. J. A Structured Decisional-Support Tool for Rapid Bioprocess Modelling. American Chemical Society 225th National Meeting, New Orleans, Louisiana, USA, Presented on 26th Mar 2003.

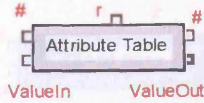
Lim, A. C.; Farid, S.; Washbrook, J.; Titchener-Hooker, N. J. The Use of a Modelling Tool to Quantify Risks and Uncertainty in the Biomanufacturing Industry. European Congress Biotechnology, Basel, Switzerland, Presented on 28th August 2003.

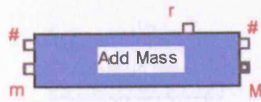
APPENDIX B

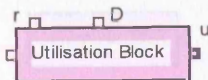
Newly Programmed Blocks

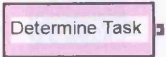
Table B.1. Newly programmed blocks in the tool.

Block Name	Block	Description
Convert		Convert all time units to hours.
<pre>// Declare constants and static variables here. integer Units; real Duration; // This message occurs for each step in the simulation. on simulate { // Determine the units used. if (Minutes) Units = 1; if (Hours) Units = 2; if (Days) Units = 3; if (Weeks) Units = 4; // Convert all units to hours. if (Units == 1) Duration = RawTaskProTime/60; if (Units == 2) Duration = RawTaskProTime; if (Units == 3) Duration = RawTaskProTime*24; if (Units == 4) Duration = RawTaskProTime*7*24; TaskProTime = Duration; TaskProTimeOut = Duration; } // If the dialog data is inconsistent for simulation, abort. on checkdata { } // Initialize any simulation variables. on initsim { }</pre>		

Block name	Block	Description
Attribute Table		Store the attributes (i.e. mass and volume) of process streams.
<pre> // Declare constants and static variables here. integer i; integer j; // This message occurs for each step in the simulation. on simulate { } // If the dialog data is inconsistent for simulation, abort. on checkdata { } // Initialize any simulation variables. on initsim { // Initialize all the rows of the table to 0 at start. for (i=0; i<32; i++) { ProcessStream[0][i] = 0; } } // Store the attribute in their respective row. On AttributeIn { ProcessStream[0][ResourceIn] = AttributeIn; } // Output the value of the attribute when required. On RowIn { AttributeOut = ProcessStream[0][RowIn]; } // Reset the attribute table. On ResetIn { for (j=0; j<32; j++) { ProcessStream[0][j] = 0; } } </pre>		

Block name	Block	Description
Add Mass		Add the mass of each individual component in the process stream.
<pre> // Declare constants and static variables here. integer i; integer j; // This message occurs for each step in the simulation. on simulate { } // If the dialog data is inconsistent for simulation, abort. on checkdata { } // Initialize any simulation variables. on initsim { // Initialize all the rows of the table to 0 at start. for (i=0; i<32; i++) { ProcessStream[0][i] = 0; } } // Add the mass of each component in the process stream. On MassIn { ProcessStream[0][ResourceIn] += MassIn; } // Output the mass of each component On RowIn { MassOut = ProcessStream[0][RowIn]; } // Reset the table after use. On ResetIn { for (j=0; j<32; j++) { ProcessStream[0][j] = 0; } } </pre>		

Block name	Block	Description
Utilisation		Plot the peak demand of resource at run time.
<pre> // Declare constants and static variables here. Real T; Real TotalUtilizn; Real LastUtiliznValue; Real CumulativeUtiliznValue; Real TempResourceAvailable; // This message occurs for each step in the simulation. on simulate { // Calculate the total utilization in a day TotalUtilizn = UtiliznIn - LastUtiliznValue; CumulativeUtiliznValue += TotalUtilizn; OutputTotalUtiliznOut = TotalUtilizn; LastUtiliznValue = CumulativeUtiliznValue; } // If the dialog data is inconsistent for simulation, abort. on checkdata { } // Initialize any simulation variables. on initsim { T = 1; LastUtiliznValue = 0; CumulativeUtiliznValue = 0; TempResourceAvailable = ResourceAvailableIn; } </pre>		

Block name	Block	Description
Determine Task		Specify the type of manufacturing process task.

```

// Declare constants and static variables here.


// This message occurs for each step in the simulation.
on simulate
{
  if (Fermentation == 1) TaskOut = 0;
  if (Centrifugation == 1) TaskOut = 1;
  if (AffinityChromatography == 1) TaskOut = 2;
  if (IonExchange == 1) TaskOut = 3;
  if (GelChromatography == 1) TaskOut = 4;
  if (Concentration == 1) TaskOut = 5;
  if (Diafiltration == 1) TaskOut = 6;
  if (ChemRetroVirus == 1) TaskOut = 7;
  if (VirusRednFiltration == 1) TaskOut = 8;
  if (FinalFiltration == 1) TaskOut = 9;
  if (CIP == 1) TaskOut = 10;
  if (SIP == 1) TaskOut = 11;
  if (Equilibration == 1) TaskOut = 12;
  if (Regeneration == 1) TaskOut = 13;
  if (ReEquilibration == 1) TaskOut = 14;
  if (PerfusionCulture == 1) TaskOut = 15;
}

// If the dialog data is inconsistent for simulation, abort.
on checkdata
{
}

// Initialize any simulation variables.
on initsim
{
}

on CreateBlock
{
  Fermentation = TRUE;
}

```

Block name	Block	Description
QC/QA		Specify if QC/QA is required.
<pre> // Declare constants and static variables here. // This message occurs for each step in the simulation. on simulate { } // If the dialog data is inconsistent for simulation, abort. on checkdata { } // Initialize any simulation variables. on initsim { if (QCQA == 1) QCQAIn = 1; if (NoQCQA == 1) QCQAIn = 0; } on createblock { NoQCQA = 1; } </pre>		

The figure below illustrates the sequence of functions in a *product-manufacture* task (e.g. fermentation). The block coding within each function is complex and consists of multiple blocks connected to perform the desired procedure. Figure B.2 shows the detail of the “Check Equipment Status” function as an illustration of the coding within each function.

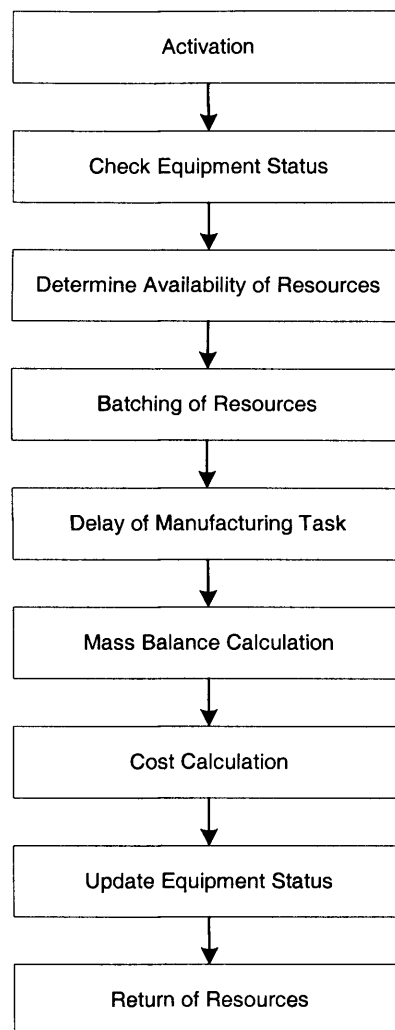


Figure B.1. Sequence of functions in a *product-manufacture* task.

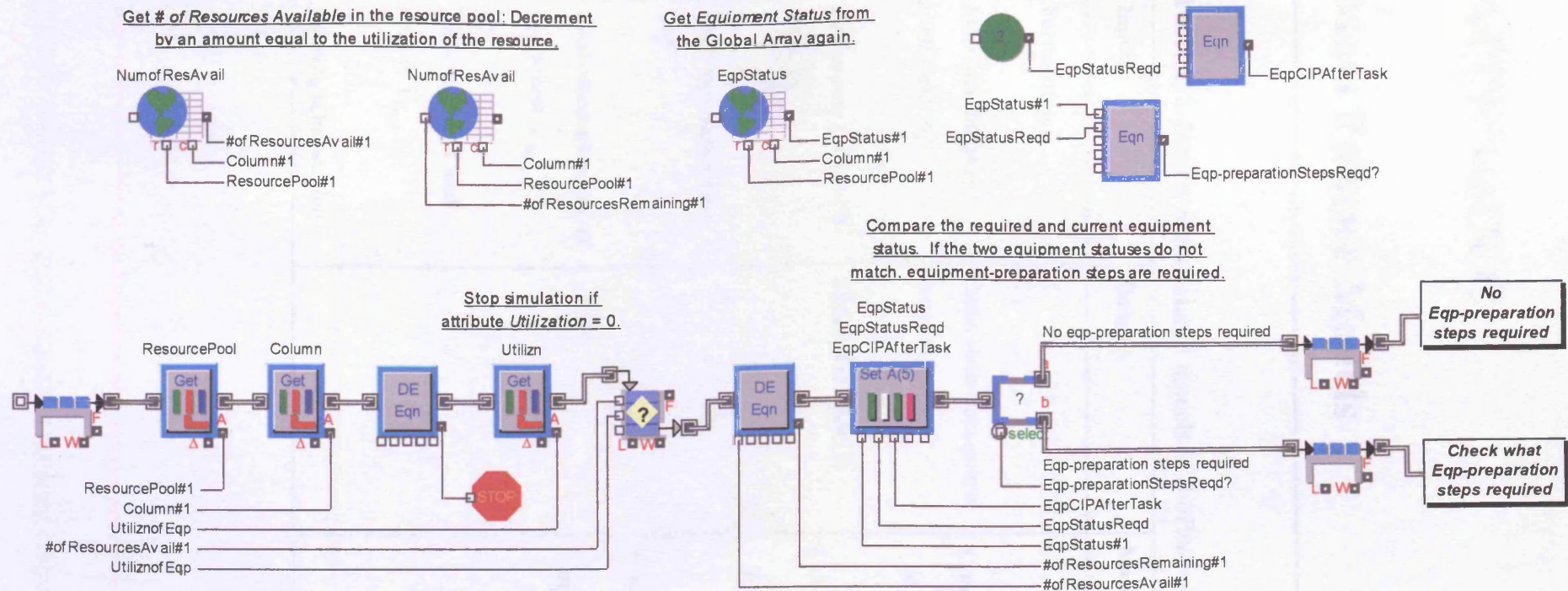


Figure B.2. Graphical diagram illustrating the blocks connected to set up the “Check Equipment Status” function within the *fermentation* task. The function compares the required and current equipment status before commencing the manufacturing process. If the two equipment statuses do not match, the relevant equipment-preparation tasks are activated.

APPENDIX C

Mass Balance Models

Table C.1. Simple mass balance models in *BioPharmkit*.*

Inputs	Outputs	Mass Balance Calculations
Fermentation		
Mass-stoichiometry coefficient, a_i	Outlet stream component mass, $m_{i\ out}$	1. $m_{out\ ref}$ $m_{out\ ref} = c_{ref} * V_{in\ tot}$
Feed stream component mass, $m_{i\ in}$	Extent of reaction, x	2. x $x = \frac{m_{in\ ref} - m_{out\ ref}}{m_{in\ lim}} * \frac{a_{lim}}{a_{ref}}$
Reference component, ref		3. $m_{i\ out}$
Final concentration of ref component, c_{ref}		$m_{i\ out} = m_{i\ in} - m_{in\ lim} * x * \frac{a_i}{a_{lim}}$
Total volume of feed stream, $V_{in\ tot}$		
Limiting substrate, lim		

Inputs	Outputs	Mass Balance Calculations
Centrifugation Solids-carry-over-fraction, S Solid-volume-fraction in sediment, $v_{s_{sed}}$ Total feed stream component masses, $m_{in_{tot}}$ Total volume of feed stream, $V_{in_{tot}}$ Solid density, ρ_s * Assumption: Only 1 solid component	Solids removal fraction, R Supernatant and sediment solid component masses, $m_{s_{sup}}, m_{s_{sed}}$ Supernatant and sediment liquid component masses, $m_{l_{i_{sup}}}, m_{l_{i_{sed}}}$	1. R $R = 1 - S$ 2. $m_{s_{sed}}, m_{s_{sup}}$ $m_{s_{sed}} = R * m_{s_{in}}$ $m_{s_{sup}} = m_{s_{in}} - m_{s_{sed}}$ 3. $\rho_{l_{in}}$ $m_{l_{in}} = m_{in_{tot}} - m_{s_{in}}$ $V_{l_{in}} = V_{in_{tot}} - V_{s_{in}}$ $\rho_{l_{in}} = \frac{m_{l_{in}}}{V_{l_{in}}}$ 4. $m_{l_{sed}}$ $V_{s_{sed}} = \frac{m_{s_{sed}}}{\rho_s}$ $V_{tot_{sed}} = \frac{V_{s_{sed}}}{v_{s_{sed}}}$ $V_{l_{sed}} = V_{tot_{sed}} - V_{s_{sed}}$ $m_{l_{sed}} = V_{l_{sed}} * \rho_{l_{in}}$ 5. $m_{l_{i_{sed}}}, m_{l_{i_{sup}}}$ $m_{l_{i_{sed}}} = \frac{m_{l_{i_{in}}}}{m_{l_{in}}} * m_{l_{sed}}$ $m_{l_{i_{sup}}} = m_{l_{i_{in}}} - m_{l_{i_{sed}}}$

Inputs	Outputs	Mass Balance Calculations
Membrane filtration Calculation mode: Membrane area per unit, A / Processing time per cycle, t Average flux, J Volume of feed stream, V_{in} No of cycles, n Concentration factor, CF Rejection coefficient, RC	Output mode: Membrane area per unit, A / Processing time per cycle, t Total processing time, T Permeate and retentate stream component masses, $m_{i\ perm}$, $m_{i\ ret}$	1. A , t $A = \frac{V_{in}(1 - CF^{-1})}{J * n * t} \text{ or }$ $t = \frac{V_{in}(1 - CF^{-1})}{J * n * A}$ 2. T $T = n * t$ 3. V_{ret} $V_{ret} = \frac{V_{in}}{CF}$ 4. $m_{i\ ret\ RC \neq 0}$ ($0 < RC_i \leq 1$) $m_{i\ ret\ RC \neq 0} = m_{i\ in} * CF^{(RC_i - 1)}$ $V_{i\ ret\ RC \neq 0} = \frac{m_{i\ ret\ RC \neq 0}}{\rho_i}$ 5. $m_{i\ ret\ RC = 0}$ ($RC_i = 0$) $V_{ret\ RC = 0} = V_{ret} - \sum V_{i\ ret\ RC \neq 0}$ $V_{i\ ret\ RC = 0} = \frac{V_{i\ in}}{V_{in\ RC = 0}} * V_{ret\ RC = 0}$ $m_{i\ ret\ RC = 0} = V_{i\ ret\ RC = 0} * \rho_i$ 6. $m_{i\ perm}$ $m_{i\ perm} = m_{i\ in} - m_{i\ ret}$

Inputs	Outputs	Mass Balance Calculations
Diafiltration Calculation mode: Membrane area per unit, A / Processing time per cycle, t Average flux, J No of cycles, n Rejection coefficient, RC No of diafiltration volumes, D Volume of components in tank, V_o Component masses in tank, m_o	Output mode: Membrane area per unit, A / Processing time per cycle, t Total processing time, T Permeate and retentate stream component masses, $m_{i\ perm}$, $m_{i\ ret}$	1. A , t $A = \frac{D * V_o}{J * n * t} \text{ or } t = \frac{D * V_o}{J * n * A}$ 2. T $T = n * t$ 3. V_{ret} $V_{ret} = V_o$ 4. $m_{i\ ret\ from\ V_o}$ $m_{i\ ret\ from\ V_o} = m_{i\ o} * e^{-D(1-RC_i)}$ $V_{i\ ret\ from\ V_o} = \frac{m_{i\ ret\ from\ V_o}}{\rho_i}$ 5. $m_{i\ ret\ from\ V_{buff}}$ $V_{ret\ from\ V_{buff}} = V_{ret} - \sum V_{i\ ret\ from\ V_o}$ $V_{i\ ret\ from\ V_{buff}} = \frac{V_{i\ buff}}{V_{buff}} * V_{ret\ from\ V_{buff}}$ $m_{i\ ret\ from\ V_{buff}} = V_{i\ ret\ from\ V_{buff}} * \rho_i$ 6. $m_{i\ ret}$ $m_{i\ ret} = m_{i\ ret\ from\ V_o} + m_{i\ ret\ from\ V_{buff}}$ 7. $m_{i\ perm}$ $m_{i\ perm} = m_{i\ in} - m_{i\ ret}$

Inputs	Outputs	Mass Balance Calculations
Chromatography		
Column height, H	Processing time per cycle, t	1. $t_r, \quad r = L, W, E$
No of column volumes, CV	Buffer volumes required, V	$t_r = \frac{H * CV_r}{u_r}$
Linear flow rates, u	Product and waste stream masses, $m_{i prod}, m_{i waste}$	2. $V_r, \quad r = L, W, E$
Column volume, V_{col}		$V_r = CV_r * V_{col} * n$
No of cycles, n		3. $V_{prod} = CV_{prod} * V_{col}$
Yield fraction, y		$m_{i prod from L} = m_{i L} * y_i$
Product-stream-column-volumes, CV_{prod}		$V_{i prod from L} = \frac{m_{i prod from L}}{\rho_i}$
		$V_{prod from E} = V_{prod} - \sum V_{i prod from L}$
		$V_{i prod from E} = \frac{V_{i E}}{V_E} * V_{prod from E}$
		$m_{i prod from E} = V_{i prod from E} * \rho_i$
		$m_{i prod} = m_{i prod from L} + m_{i prod from E}$
		$m_{i waste} = m_{i L} + m_{i W} + m_{i E} - m_{i prod}$
		From gel filtration, the equations were similar except there were no terms related to the wash step.

Inputs	Outputs	Mass Balance Calculations
<p>Dead-end filtration</p> <p>Calculation mode: Membrane area per unit, A / Processing time per cycle, t</p> <p>Average flux, J</p> <p>Rejection factor, RF</p> <p>Particle-volume fraction, $v_{p\ ret}$</p>	<p>Output mode: Membrane area per unit, A / Processing time per cycle, t</p> <p>Permeate and retentate stream component masses, $m_{i\ perm}$, $m_{i\ ret}$</p>	<p>1. $m_{i\ ret} (0 < RF_i \leq 1)$</p> $m_{i\ ret\ RF \neq 0} = RF * m_{i\ in}$ $V_{i\ ret\ RF \neq 0} = \frac{m_{i\ ret\ RF \neq 0}}{\rho_i}$ <p>2. $m_{i\ ret\ RF=0} (RF_i = 0)$</p> $V_{ret} = \frac{\sum V_{i\ ret\ RF \neq 0}}{v_{p\ ret}}$ $V_{ret\ RF=0} = V_{ret} - \sum V_{i\ ret\ RF \neq 0}$ $V_{i\ ret\ RF=0} = \frac{V_{i\ in}}{V_{in\ RF=0}} * V_{ret\ RF=0}$ $m_{i\ ret\ RF=0} = V_{i\ ret\ RF=0} * \rho_i$ <p>3. $m_{i\ perm}$</p> $m_{i\ perm} = m_{i\ in} - m_{i\ ret}$ <p>4. A, t</p> $V_{perm} = V_{in} - V_{ret}$ $A = \frac{V_{perm}}{J * t} \text{ or } t = \frac{V_{perm}}{J * A}$
<p>Viral clearance</p> <p>No of virus units in inlet stream, vr_{in}</p> <p>Log clearance factor, LCF</p>	<p>No of virus units in outlet stream, vr_{out}</p>	<p>1. vr_{out}</p> $vr_{out} = vr_{in} * 10^{LCF}$

* Adapted from Farid (2001)

Nomenclature

Symbols

a	Mass-stoichiometry coefficient
A	Membrane area
c	Concentration
C	Cost of equipment
C_e	Equipment purchase cost
C_f	Fixed capital investment
CF	Concentration factor
CV	Number of column volumes
D	Number of diafiltration volumes
f_L	Lang factor
H	Column height
J	Average flux
LCF	Log clearance factor
m	Mass
n	Number of cycles
NCF	Net cash flow
NPV	Net present value
PV	Present value
Q	Capacity
r	Discount rate
R	Solid removal fraction
RC	Rejection coefficient
RF	Rejection fraction
S	Solids carry-over fraction
t	Processing time per cycle
T	Total processing time
u	Linear velocity
v	Volume fraction
vr	Virus units
V	Volume
x	Extent of reaction
X	Random variable
y	Component yields
ρ	Density
σ	Standard deviation
μ	Mean

Subscripts

<i>buff</i>	Buffer
<i>col</i>	Column
<i>E</i>	Elution
<i>i</i>	Component
<i>in</i>	Inlet stream
<i>l</i>	Liquid phase
<i>L</i>	Load
<i>lim</i>	Limiting substrate
<i>out</i>	Outlet stream
<i>p</i>	Particle
<i>perm</i>	Permeate
<i>prod</i>	Product stream
<i>ref</i>	Reference component
<i>ret</i>	Retentate
<i>s</i>	Solid
<i>sed</i>	Sediment
<i>sup</i>	Supernatant
<i>tot</i>	Total
<i>W</i>	Wash
<i>waste</i>	Waste